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THE EFFECT OF 2-PIVALYL-1,3-INDANDIONE ON BODY LICE, WHEN ADMINISTERED ORALLY TO RABBIT HOSTS[1]

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That bloodsucking arthropod parasites can be killed by internal medication of the host is now well established. Lindquist *et al.* (1944) obtained a high kill of bed bugs, *Cimex lectularius* L. and *C. hemipterus* F., fed on rabbit hosts that had received large doses of DDT, pyrethrum, and a mixture of pyrethrum and N-isobutyl-undecylenamide. In replications a few months later they obtained no kill. No explanation for the discrepancy was offered. Meillon (1946) found that the gamma isomer of benzene hexachloride, when given internally to rabbit hosts, was toxic to *C. lectularius;* the yellow-fever mosquito, *Aedes aegypti* (L.); and the relapsing-fever tick, *Ornithodoros moubata* Wheeler. Knipling *et al.* (1948) found certain toxicants that were effective against the body louse, *Pediculus humanus corporis* Deg., *Aedes aegypti* mosquitoes; the ear mite, *Psoroptes equi* var. *cuniculi* Delafond; and the lone star tick, *Amblyomma americanum* (L.), when administered to the rabbit host either orally or by injection. Mocsy (1947) controlled scabies in dogs and swine and lice in swine and cattle by peroral application of certain contact poisons. Daily dosages of 0.05 to 0.07 gm./kg. were given for 4 days to obtain control. After a single dose the drug was detectable in the blood for 7 to 8 days. Garnham (1947) reported that the minimum lethal dosage of gamma benzene hexachloride administered orally to rabbits was between 25 and 30 mg./kg. for *Aedes aegypti.* After a single dose at the rate of 40 mg./kg. the blood remained toxic 2 to 4 days, but the dose was usually fatal to the host. Although Knipling *et al.* (1948) showed that smaller repeated doses of toxicant were effective, they did not determine the minimum daily dose required to maintain a high toxic level in the blood of the host.

This paper gives the results of experiments undertaken with 2-pivalyl-1,3-indandione, one of the more effective compounds used by Knipling *et al.* (1948), at the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine. The objectives of these experiments were to determine (1) the minimum quantity of the chemical required in the diet of rabbits to prevent reproduction of body lice feeding on the rabbit, (2) the variation in susceptibility to the chemical of various stages of the test insect, and (3) the length of time the blood remains toxic after ingestion of the chemical ceases. 2-Pivalyl-1,3-indandione (also called tertiary

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butyl valone) is known to be toxic to warm-blooded animals at fairly low doses (Kabat *et al.* 1944), and was selected for experimental purposes only. The material used was a commercial product having a melting point of 107°–109.5°C.

MATERIALS AND METHODS

A common mixed breed of domesticated rabbit was employed as the host in all tests. Three rabbits were fed a diet of commercial rabbit pellets to which sufficient 2-pivalyl-1,3-indandione had been added so that the desired dose was contained in 50 grams of feed. The chemical, dissolved in acetone, was added to the pellets and the solvent allowed to evaporate in the air, as described by Knipling *et al.* (1948).

The rabbits readily consumed the treated food and, after it was eaten, were given enough untreated pellets to make up the total daily food requirements. The lice, *Pediculus humanus corporis,* were reared in the laboratory on rabbits according to the method described by Culpepper (1946).

EXPERIMENTAL

Minimum Effective Dose of 2-Pivalyl-1,3-Indandione.—After the three rabbits had been established as suitable hosts for body lice, they were fed the treated pellets, each one receiving a different dose. Forty-eight hours after the treatment was started or, in later experiments, after the dosage was increased, 400 newly hatched louse nymphs were placed on each rabbit for an initial blood meal and were allowed two blood meals daily during the next 20 days. The survivors were counted at 24-hour intervals. If any of the lice survived long enough to lay eggs, the dose was considered insufficient and the quantity of chemical was increased. Egg production usually started on the 11th or 12th day among the surviving females, and no difference in viability of the eggs was noted. Results of these experiments are shown in table 1.

TABLE 1.—*Survival and egg production of lice fed twice daily on rabbits treated with different daily doses of 2-pivalyl-1,3-indandione. The experiments were started with 400 newly hatched nymphs.*

Rabbit No.	Daily dosage (mg./kg.)[1]	Percentage[2] of survivors after indicated number of days—									Total number of eggs	Average number of eggs per female per day
		1	2	3	4	5	10	12	15	20		
1	None	99	99	98	96	93	80	74	72	66	6,468	3.9
2	None	97	96	94	92	89	81	80	73	39	3,171	3.1
3	None	98	93	90	88	86	80	75	70	45	4,312	3.9
1	0.02	..	..	..	..	99	93	90	85	61	6,820	4.1
1	.025	..	..	..	..	98	92	91	82	71	5,781	3.8
2	.03	..	..	..	..	99	96	95	90	78	6,829	4.3
3	.035	..	..	..	..	97	95	95	92	76	6,687	4.1
1	.05	..	..	..	..	98	93	93	88	63	5,802	4.6
3	.075	..	..	..	..	96	91	89	74	29	3,195	3.1
2	.10	..	..	..	..	97	93	92	74	30	2,423	2.5
1	.125	..	..	..	..	99	56	42	6	2	123	.4
1	.15	62	5	0	..	..	..	..	..	..	....	..
3	.175	76	11	0	..	..	..	..	..	..	....	..
2	.20	70	16	1	0	..	..	..	..	..	....	..

[1] The original weights of the rabbits were: No. 1, 3.63 kg.; No. 2, 2.38 kg.; No. 3, 2.16 kg.
[2] The percentages in the last 3 experiments are based on 3 replications with 400 lice each.

Susceptibility of Different Stages of Lice.—The difference in susceptibility to the chemical between the several stages and between different lots of lice was determined by giving 100 each of first-, second-, and third-instar nymphs and 50 adults of each sex a single blood meal on the rabbits receiving 0.15, 0.175, and 0.20 mg./kg. The

survivors were counted 24 hours later. Seven lots of each stage were used, and statistically significant differences in the susceptibility of these lots and stages were found. The percentage survival of these lice is shown in table 2.

TABLE 2.—*Percentage survival of different stages of lice 24 hours after one feeding on rabbits treated with different dosages of 2-pivalyl-1,3-indandione. (7 replications with 100 nymphs of each instar and 50 adults of each sex.)*

Stage	0.15 mg./kg.	0.175 mg./kg.	0.20 mg./kg.	Mean for all dosages
First instar	42 ± 12.1	43 ± 10.0	49 ± 9.1	44
Second instar	49 ± 8.08	61 ± 9.0	57 ± 7.4	55
Third instar	57 ± 5.7	71 ± 5.7	68 ± 3.3	66
Female	7 ± 2.1	8 ± 2.6	7 ± 1.2	7
Male	39 ± 7.5	44 ± 6.0	51 ± 6.0	45

Duration of Toxicity.—After the feeding of the 2-pivalyl-1,3-indandione was discontinued, 50 females were placed daily on each rabbit to determine the length of time the blood of the host remained toxic. Knipling *et al.* (1948) found that, following the ingestion of 0.1 mg./kg. of the chemical daily for 18 days, the blood of the rabbit was toxic to female lice for about 30 days after the treatment was discontinued. However, in these tests the blood remained toxic for only 3 days. No explanation is offered for these differences.

Toxicity to the Host.—Rabbit No. 1 died the second day after feeding of the chemical was discontinued. The animal was autopsied and various tissues were examined pathologically.[2] The pathologists concluded that death was caused primarily by an advanced amyloid nephrosis, which was probably not attributable to 2-pivalyl-1,3-indandione although the chemical had been fed continuously for 8 months. The animal was more than 4 years of age and was of some historic interest in that it was the first rabbit found at this laboratory to be a suitable host for body lice.

DISCUSSION

Investigators previously referred to (Knipling *et al.* 1948) obtained up to 85 percent mortality of adult lice allowed repeated meals on a rabbit receiving 0.1 mg. of 2-pivalyl-1,3-indandione per kilogram daily. In our experiments the minimum level required to prevent development of one generation was about 0.15 mg./kg., and there was a very marked drop in the length of survival between this dosage and the next lower one (0.125 mg./kg.)

With dosages up to 0.1 mg./kg. the percentage of lice reaching maturity (10 to 12 days) was considerably higher than among the lice feeding on the untreated rabbits. The number of eggs per female louse was also somewhat higher for the treated animals until the 0.1 mg./kg. dosage was reached, when there was a definite reduction.

Of the lice given only one blood meal on a treated animal (table 2), much the highest mortality occurred among the females, which took the larger quantity of blood. On the other hand, the nymphs showed an increasing survival rate with successive instars. The average percentage survival of the different stages on the three rabbits was not proportional to the treatment dosages, probably owing to individual host variation.

[2] The histopathological examination was made by A. A. Nelson, of the Food and Drug Administration.

SUMMARY

Laboratory experiments were conducted at Orlando, Fla., to determine the effect of 2-pivalyl-1,3-indandione on body lice, *Pediculus humanus corporis* Deg., when administered orally to rabbit hosts. Three rabbits were fed a diet of commercial rabbit pellets to which sufficient 2-pivalyl-1,3-indandione had been added so that the desired dose was contained in 50 grams of feed.

The minimum quantity of 2-pivalyl-1,3-indandione necessary in the daily diet of rabbit hosts to prevent reproduction of body lice feeding on the rabbits was about 0.15 mg. of chemical per kilogram of body weight. At this dosage young nymphs allowed two blood meals daily succumbed within 3 days. At a dosage of 0.125 mg./kg. about half the lice reached maturity and a few viable eggs were deposited.

Among lice given a single blood meal on rabbits receiving 0.15, 0.175, and 0.20 mg. of 2-pivalyl-1,3-indandione per kilogram, the mortality was much higher for females than for the other stages. The nymphs showed a decreasing mortality as they became older.

The blood of the rabbits remained toxic only 3 days after ingestion of the chemical ceased.

One rabbit which had received varying quantities of 2-pivalyl-1,3-indandione over a period of 8 months died. No lesions definitely attributable to the chemical were found upon autopsy.

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A STUDY ON THE COMPARATIVE SUSCEPTIBILITY OF SNAIL VECTORS TO STRAINS OF *SCHISTOSOMA MANSONI*

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Several investigators have reported that snail vectors of schistosomes of man vary in their susceptibility to infection. Vogel (1942) found *Planorbis pfeifferi* (*Biomphalaria pfeifferi*) of West African origin to be a more susceptible host than *Planorbis guadaloupensis* (*Australorbis glabratus*) of Dutch Guiana origin when a West African strain of *Schistosoma mansoni* was employed. Stunkard (1946) and Cram, Files, and Jones (1947) found that *Planorbis boissyi* (*Biomphalaria boissyi*) of Egyptian origin was refractory to the *S. mansoni* strain of the West Indies. Cowper (1947) reported *Bulinus truncatus* of Egyptian origin to be completely "unattractive" to miracidia of a West African strain of *S. haematobium*. It appeared desirable to extend these observations on the relative susceptibility of certain species of snails that serve as intermediate hosts to *S. mansoni*. In the present study, the snail vectors of *S. mansoni* from Liberia, Egypt, and three countries of the Western Hemisphere were tested for their susceptibility to several strains of the parasite.

MATERIALS AND METHODS

A. glabratus from Puerto Rico, Venezuela, and Brazil, *B. pfeifferi* from Liberia, and *B. boissyi* from Egypt were employed in these experiments. The *A. glabratus* were obtained from laboratory-reared colonies established from eggs or adult specimens of the snail vector that had been received from the following sources: Puerto Rico, three lots from San Juan, 1943, 1946, 1947; Venezuela, one lot from Caracas, 1946, and one lot from Lake Valencia, 1947; Brazil, two lots from Recife, 1946 and 1947. The *B. pfeifferi* and the *B. boissyi* were drawn from laboratory-reared colonies established from adult specimens received from Monrovia, Liberia, 1948 and from Cairo, Egypt, 1947, respectively.

All three species of snails were identified by Dr. Elmer G. Berry of this Laboratory. In the past there has been some question as to whether the *Australorbis* serving as intermediate host for *S. mansoni* in Puerto Rico, Venezuela, and Brazil are all representatives of the same species. Scott (1940) made an extended study of the *Australorbis* from Venezuela and Brazil and he concluded that no conchological or anatomical differences could be determined in the *Australorbis* from these localities. Pilsbry was in agreement with his views. In addition, Dr. Berry has made a comparative study of the shell characters, gross anatomy, and genitalia of the *Australorbis* from Puerto Rico, Venezuela, and Brazil, used in this study, and has found no evidence to indicate that any of these specimens are other than *Australorbis glabratus* (Say).

Strains of *S. mansoni* from Puerto Rico, Venezuela, and Egypt, and a Brazilian-Puerto Rican parasite cross strain were used for exposure of the snail vectors. The

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Puerto Rican strains were secured from several sources—monkeys, rodents, and humans. *S. mansoni* of Venezuelan origin was derived from infected mice received from Caracas, Venezuela and later established in mice, hamsters, and a monkey. The Egyptian strain was established in rodents and a monkey from infected *B. boissyi* received from Cairo, Egypt. The Brazilian-Puerto Rican parasite cross strain was obtained by exposing mice to cercariae (known to produce female schistosomes) from an *A. glabratus* infected with *S. mansoni* of Brazilian (Recife) origin and to cercariae (known to produce male schistosomes) from an *A. glabratus* infected with *S. mansoni* of Puerto Rican origin.

Two methods of exposure were employed in the experiments. Snails were exposed either individually in Syracuse dishes to small numbers of miracidia, 3 to 6 miracidia per specimen, or were exposed en masse in finger bowls to large numbers of miracidia, 100 miracidia per 20 specimens. Tests were performed in a series of small experiments so that lots of 10 to 30 snails were exposed at one time to miracidia from a single culture. An attempt was made to include proportionate numbers of specimens of various age groups from young juveniles to adults in each of the many experiments. Miracidia for the exposures were obtained from cultures of liver or fecal pellets of hamsters and from monkey and human feces. Exposed snails were maintained in small aquaria which contained gravel and greenery, were fed lettuce and fish food, and were kept at room temperature for the prepatent period.

The aquaria containing the exposed snails were checked daily for the emergence of cercariae from the twenty-fifth day of the prepatent period. Upon evidence of infection, or on the twenty-eighth day after exposure if cercariae had not been shed, the snails were isolated in test tubes. The isolated specimens were then observed daily for one week. Snails which still remained negative for infection at the end of this period were returned to the original aquaria which in turn were checked twice weekly for two weeks for possibly delayed infections. Specimens which survived for 49 days without shedding cercariae were discarded and recorded as negative. There was some mortality of snails during the prepatent, isolation, and observation periods. However, only specimens which died after the twenty-fifth day of exposure were dissected and examined for sporocysts and cercariae, and these snails were included in tabulations of the number of snails positive or negative for infection. All snails which died previous to the twenty-fifth day of the prepatent period were discarded and were tabulated as "undetermined."

RESULTS

A total of 1,865 *A. glabratus,* 204 *B. pfeifferi,* and 431 *B. boissyi* were exposed to various strains of *S. mansoni.* The results of the exposures, which are summarized in table 1, revealed that the Puerto Rican *A. glabratus* became infected with all strains of the parasite; 46 per cent became infected with the Puerto Rican animal strain, 38 per cent with the Puerto Rican human strain, 44 per cent with the Venezuelan strain, 37 per cent with the Brazilian-Puerto Rican cross strain, and only 9 per cent with the Egyptian strain.

Venezuelan *A. glabratus* likewise became infected with the several strains of *S. mansoni* tested, although they showed a higher rate of infection with the Brazilian-Puerto Rican cross strain than with the Venezuelan strain. Here again, the percentage of specimens infected with the Egyptian strain was low, 7 per cent.

TABLE 1.—*Results following the exposure of* Australorbis glabratus, Biomphalaria pfeifferi, *and* Biomphalaria boissyi *to various strains of* Schistosoma mansoni.

	Australorbis glabratus from Puerto Rico				*Australorbis glabratus* from Venezuela				*Australorbis glabratus* from Brazil				*Biomphalaria pfeifferi* from Liberia				*Biomphalaria boissyi* from Egypt			
Strain and source of *S. mansoni*	T	+	−	U	T	+	−	U	T	+	−	U	T	+	−	U	T	+	−	U
Puerto Rican Experimental Animals	244	113 *46*	94 *39*	37 *15*	143	47 *33*	65 *46*	31 *21*	144	0	132 *91*	12 *9*	70	27 *38*	28 *40*	15 *22*	60	0	43 *71*	17 *29*
Puerto Rican Human source	114	43 *38*	51 *45*	20 *17*	25	4	16	5	92	6 *7*	82 *89*	4 *4*					39	0	30	9
Venezuelan Experimental Animals	158	68 *44*	59 *37*	31 *19*	122	28 *23*	59 *48*	35 *29*	125	0	115 *92*	10 *8*	72	47 *65*	1 *14*	24 *21*	70	0	50 *71*	20 *29*
Brazilian-Puerto Rican Experimental Animals	113	42 *37*	62 *54*	9 *9*	101	46 *46*	38 *38*	17 *16*	141	68 *41*	64 *45*	9 *14*	30	9	12	9	90	0	73 *81*	17 *19*
Egyptian Experimental Animals	134	13 *9*	95 *72*	26 *19*	96	7 *7*	80 *83*	9 *10*	113	0	107 *95*	6 *5*	32	6	9	17	122	36 *30*	58 *47*	28 *23*

T = Total number of specimens exposed.
+ = Number of specimens positive for *S. mansoni.*
− = Number of specimens negative for *S. mansoni.*
U = Undetermined (specimens that died before the twenty fifth day of the prepatent period).
Numbers in italics = Percentages (Omitted in instances where the total number of snails exposed was small).

The Brazilian *A. glabratus,* however, could not be infected with all *S. mansoni* strains. Only 7 per cent of the snails were infected with the Puerto Rican *S. mansoni* of human source, and none was infected with the Puerto Rican strain from experimental animals, the Venezuelan strain, nor the Egyptian strain. On the other hand, 41 percent of the Brazilian *A. glabratus* shed cercariae following exposure to the hybrid Brazilian-Puerto Rican cross strain.

B. pfeifferi from Liberia was readily infected with four strains, the Puerto Rican experimental animal (38 per cent), the Venezuelan (65 per cent), Brazilian-Puerto Rican cross, and the Egyptian strain. Percentages of infection for the last two strains were not determined because of the limited number of specimens exposed in these instances.

The *B. boissyi* specimens were refractory to the Puerto Rican, Venezuelan, and the Brazilian-Puerto Rican cross strains, but 30 per cent of these snails became infected when the Egyptian strain of *S. mansoni* was employed.

DISCUSSION

The observations recorded in the previous section contribute additional information on the relationship between snail vector and parasite in the life cycle of *S. mansoni.* These experiments show that snail vectors from some endemic areas are capable of serving as intermediate hosts for several strains of *S. mansoni,* whereas other vectors are not so versatile. A graphic representation of the differences is shown in Figure 1.

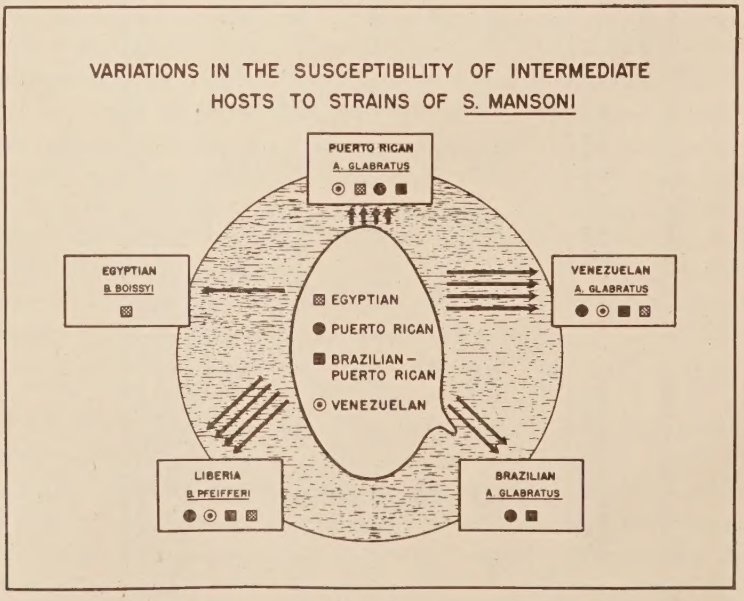

FIG. 1

The *A. glabratus* from Puerto Rico and Venezuela were readily infected with the Puerto Rican, Venezuelan, and the Brazilian-Puerto Rican cross strains of the parasite. These snails were also susceptible, but to a lesser extent, to the Egyptian strain. The susceptibility of this species has also been observed by other investi-

gators, e.g., Vogel (1942) who found *A. glabratus* from Dutch Guiana a suitable host for a West African and Brazilian strain of the parasite. Cowper (1947) also was able to infect this snail of presumed South American or West Indian stock with a Uganda strain of *S. mansoni.*

However, even within this species, differences in susceptibility to strains of *S. mansoni* are exhibited. *A. glabratus* from Brazil (Recife) was refractory to most foreign strains of the parasite, but was readily susceptible to the hybrid strain that had been developed from female schistosomes of Brazilian origin (Recife) and male schistosomes of Puerto Rican origin. Although the *Australorbis* from the three countries, Puerto Rico, Venezuela, and Brazil, must be considered the same species on the basis of morphological criteria, it appears that there is a physiological difference between *A. glabratus* from Puerto Rico and Venezuela, and *A. glabratus* from Brazil.

B. pfeifferi from Liberia also seems to be a favorable vector for many strains of the parasite, namely, the Puerto Rican, Venezuelan, Brazilian-Puerto Rican cross, and the Egyptian strains. In addition, Vogel (1942) was able to infect this species with *S. mansoni* from West Africa and Brazil. In contrast, *B. boissyi* from Egypt was refractory to all the strains of *S. mansoni* from the Western Hemisphere and susceptible only to the Egyptian strain. As noted, Cram, Files, and Jones (1947) were unable to infect specimens of *B. boissyi* with a Puerto Rican strain of the parasite and Stunkard (1947) also found this species refractory to the same strain.

These variations in susceptibility for strains of *S. mansoni* as exhibited by the different species of vectors give further evidence as to the possible mode of spread of the disease. Most investigators believe that *S. mansoni* was brought to the Western Hemisphere from West Africa by the importation of slaves and became adapted to a new snail vector, *A. glabratus.* The susceptibility of *B. pfeifferi,* the intermediate host of *S. mansoni* in West Africa, for the parasite strains of the Western Hemisphere and the successful infection of *A. glabratus* with a West African strain of the parasite (Vogel) give further support to this theory.

There is, in addition, some evidence that the *S. mansoni* endemic in Egypt is physiologically different from the *S. mansoni* of the Western Hemisphere as indicated by the inability to infect *B. boissyi* with the West Indian, Venezuelan, or the Brazilian-Puerto Rican hybrid strains and the limited receptivity of *A. glabratus* for the Egyptian strain. From the information now available it is suggested that, on the basis of intermediate host preference, the *S. mansoni* of the Western Hemisphere is more closely related to the West African strain than to the Egyptian strain of *S. mansoni.*

SUMMARY

In this study *Australorbis glabratus* from Puerto Rico, Venezuela, and Brazil, *Biomphalaria pfeifferi* from Liberia, and *Biomphalaria boissyi* from Egypt were exposed to strains of *Schistosoma mansoni* from Puerto Rico, Venezuela, and Egypt, and a Brazilian-Puerto Rican parasite cross strain. Striking differences in the susceptibility of snail vectors for the various strains of *S. mansoni* were observed. It is suggested, on the basis of the intermediate host preference, that *S. mansoni* endemic in one area may be physiologically distinct from *S. mansoni* endemic in another area.

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PLASMODIUM OTI AND *P. HEXAMERIUM*

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Plasmodium oti was described by Wolfson (1936) from an Eastern screech owl (*Otus asio naevius*), and was studied by her both in this host and in the canary. Its characteristics included the formation of elongate gametocytes, the usual production of eight merozoites per segmenter, granular or rod-like pigment, and failure to displace the host-cell nucleus in any stage. These and other features are summarized in Table 1.

Although the screech owl from which the species was isolated had a mixed infection, the second species (identified as *P. subpraecox*) was observed in only one of the six canaries inoculated, and since it produced both round gametocytes and round schizonts it was easily distinguished from *P. oti*. It appears from the original description that *Plasmodium oti* produced similar infections and exhibited similar morphological characteristics in both host species.

Canaries carrying *Plasmodium oti* were given the author through the courtesy of Dr. Wolfson, and the species was maintained in this host for a number of years in the author's laboratory, until service in the army terminated further research for the time being. Since the other small species of avian plasmodia were also being maintained it was possible to study and carefully compare *P. oti* with them, and especially with *P. hexamerium,* which it appeared to closely resemble.

The following comparative table is based on the original description of each of these two species, and is intended to bring out the fact that even here few differences were indicated.

TABLE 1.—*Comparative morphology of* Plasmodium oti *and* P. Hexamericum *in the vertebrate host*

Characteristic	*P. oti* (Wolfson, 1936)	*P. hexamerium* (Huff, 1935)
Trophozoites	not specifically mentioned	"amoeboid or not amoeboid" (depending on age of parasite)
Schizonts	"elongate; usually parallel to long axis of host cell"	"no longer than nuclei of erythrocytes"
Number of merozoites per segmenter	"usually eight"	"6 merozoites; rarely 8"
Gametocytes	"elongate, tending to bend around both poles of nucleus"	"elongate, extending from pole to pole of erythrocyte, ends curved"
Pigment	"granular to rod-like"; clumped in young gametocytes and schizonts, scattered in older gametocytes.	"clumped in older trophozoites and schizonts; granules large, dispersed, bacilloid in gametocytes"
Effect on host cell	nucleus not displaced	nucleus not displaced
Type of cell invaded	not definitely stated	"all stages in erythrocytes"
Length of asexual cycle	unknown	unknown (segmentation peaks 8 to 9 A.M.; synchronicity low; may be tertian or quartan)
Vector	unknown	unknown
Type host	Eastern screech owl	Blue bird

Reference to the table will show the chief differences in the species to be the average numbers of merozoites produced per segmenter, and even here there is a definite over-lapping. If this difference were a consistent one it would, of course, be of significance though hardly a sufficient basis by itself for differentiating species.

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* From the Department of Zoology, Syracuse University. Some of the observations on which this paper is based were made by Miss Elizabeth Fortney.

It is well known that strains within a species may differ somewhat in this respect. For example, *Plasmodium vivax* is stated by Manson (Manson-Bahr, 1931) to produce an average of 18 to 20 merozoites per segmenter, by Boyd (from Stitt) "15 or more," by Wilcox "16 (the) common number," and by Wenyon "about 16." In the author's experience, as stated in an earlier publication (Russell, West and Manwell) 16 is the most commonly observed number. Another reason for regarding relatively small differences in merozoite production as rather unimportant is the known fact that, in avian malaria at least, the average number of merozoites per segmenter falls considerably in the later stages of infection in some species.

Comparison of the figures of *Plasmodium oti* and *P. hexamerium,* as given in the original descriptions, shows two other differences; *P. oti* is usually lateral in the host cell, whereas *P. hexamerium* is generally polar, except (in each case) for the gametocytes. Although nothing is said in the text about it, the sexual stages of *P. hexamerium* appear to be considerably narrower than their *P. oti* counterparts.

Now it is quite possible that there are differing strains within each of these species, but in our experience *P. oti* and *P. hexamerium* are indistinguishable in blood films, and although we have had only the original strain of *P. oti* we have had a number of strains of *P. hexamerium.* We have found the usual number of merozoites per segmenter to be six in each case, with a range of from four to eight. The gametocytes also present no consistent differences. We have likewise been unable to find consistent differences in the position of the parasites within the host cell. The polar position seems both common, and about equally observed, in both species. That differences are few and relatively slight is clearly shown by the microphotographs in the accompanying plate.

Plasmodium hexamerium and *P. oti* also produces very similar infections in the canary. In both, the infection is typical of the group of small species (*hexamerium, oti, vaughani, rouxi, nucleophilum* and probably *polare*) to which each belongs. There is no sharply marked peak or crisis. Rather, there is usually a rather prolonged incubation period (although blood inoculated infections vary in this respect, according to the numbers of parasites present in the inoculum), with a rather gradual rise in parasitemia, and then an even more gradual drop, but with a sufficient number of parasites remaining in the peripheral blood to be easily demonstrable during the entire infection which, in canaries at least, is usually for the life of the bird. The character of the *P. hexamerium* infections observed in wild birds indicates that they also follow a similar pattern.

Neither species appears to be particularly pathogenic, at least in the canary. No doubt strains differ in this respect, and only the original strain of *P. oti* has been available for study. However the six or more strains of *P. hexamerium* which the author has isolated and studied at different times have all produced notably benign infections.

Both species are also alike in the relative lack of synchronicity exhibited in the asexual cycle. The length of the cycle of *P. oti* has not been studied, but that of *P. hexamerium* was believed by Huff to be tertian or quartan, although there was evidence of a daily peak in the number of reproducing forms at about 9 A.M. A study of another strain in the author's laboratory gave similar results.

Nothing is yet known about the possible occurrence of exoerythrocytic stages in either species, or their reaction to antimalarial drugs. Nor is anything known of

their physiology or biochemistry. There is therefore the possibility of differences here.

Plasmodium hexamerium appears to be fairly common in nature. Huff, in his original description, noted its occurrence in four host species (Maryland yellowthroat; catbird; bluebird; mourning dove). Jordan (1943) reports finding it in four additional species: brown thrasher, twohee, purple grackle, and robin. Herman (1938) observed it in song sparrows. In this last species of host it seems to be especially common, for the author has also seen it in 8 of 120 birds examined. He has also found it in Savannah sparrows (1 of 13), white crowned sparrows (1 of 52), white throated sparrows (1 of 12), swamp sparrows (1 of 6), Lincoln's sparrow (1 of 2), and in cowbirds (2 of 87), redwinged blackbirds (2 of 177), and in Northern yellowthroats (1 only seen). From these figures it is apparent that *P. hexamerium* occurs in many host species, and that it may be extremely common in some, although the number of birds examined is in many cases too small to give significant infection rates.

Plasmodium oti has so far been observed, apparently, only on a single occasion and in the one species of host. Nevertheless it has been recognized as probably a valid species by Brumpt (1938), Giovannola (1939), and Hewitt (1940). Of these authors, it is likely that only Hewitt had seen it. Brumpt simply quoted from the original description, and Giovannola did likewise, though he also gave a color plate. But his figures are obviously copied, with several very slight changes, from Wolfson's, though with the addition of color. The author questioned the validity of *Plasmodium oti* in two previous publications (1938; 1946). There are undoubtedly numerous characteristics, other than the morphology of the parasites in the blood of the vertebrate, which are of significance in the recognition of species of malarial plasmodia. But it seems to the author that a species should exhibit characteristics in the blood film which are sufficiently unique for ready identification if it is to be accepted as valid (Manwell, 1936). This condition *Plasmodium oti* does not seem to meet, although it is by no means certain that, with further study, other significant differences will not be discovered, possibly resulting in the demonstration of it as a subspecies or a variety.

CONCLUSIONS

Plasmodium hexamerium and *P. oti* have been carefully studied in the laboratory, and found essentially similar in morphology, type of infection produced in the canary, and in other respects. It is therefore concluded that *Plasmodium oti* should be regarded as a synonym of *Plasmodium hexamerium.*

A number of new host species are also reported for the latter species.

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EXPLANATION OF PLATE

EXPLANATION: The first and third columns (Figures 1 to 4, and 9 to 12) are microphotographs of *Plasmodium hexamerium,* and the second and fourth columns (Figures 5 to 8, and 13 to 16) show corresponding stages of *Plasmodium oti.* Magnification × 1800.

FIGS. 1 and 5. Rings.

FIGS. 2 and 6. Schizonts. The somewhat diagonal positions of the parasites are rather characteristic.

FIGS. 3 and 7. Also schizonts. Laterally placed parasites of this stage are somewhat less frequently seen than the obliquely polar forms.

FIGS. 4 and 8. Segmenters forming four merozoites.

FIGS. 9 and 13. Presegmenters destined (probably) to form six merozoites.

FIGS. 10 and 14. Segmenters with six merozoites (the usual number).

FIGS. 11 and 15. Segmenters forming eight merozoites.

FIG. 15. Shows a rather unusual arrangement.

FIGS. 12 and 16. Macrogametocytes. These vary somewhat in size, but when mature the pigment is usually scattered, as in the forms shown.

(All the microphotographs were made by Miss Stella Zimmer, of the Syracuse University College of Medicine.)


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SANGUINICOLA HURONIS N. SP. (TREMATODA: SANGUINICOLIDAE) FROM THE BLOOD SYSTEM OF THE LARGEMOUTH AND SMALLMOUTH BASSES[1]

Jacob H. Fischthal

During a parasite survey of northwest Wisconsin fishes, a species of blood fluke of the genus *Sanguinicola* Plehn, 1905, was reported by the author (1947) from the mesenteric blood vessels of the largemouth bass, *Huro salmoides* (Lac.) and the northern smallmouth bass, *Micropterus d. dolomieu* Lac. In the 1947 paper, without naming or describing the fluke, it was declared a new species because it differed morphologically from the single known North American species, *Sanguinicola occidentalis* Van Cleave and Mueller, 1932, from the heart of the walleye, *Stizostedion vitreum.*

The genus *Sanguinicola* was established by Plehn (1905) to receive *S. armata* and *S. inermis* from European cyprinids; she called them endoparasitic Turbellaria. Later (1908), she reviewed her earlier conclusions and reclassified the two species as Cestodaria. Odhner (1911) correctly placed these forms in the Trematoda and discussed his and Looss' observations on the life cycle of *S. inermis.* The life cycle of this species was further clarified by Scheuring (1922). Woodland (1923) described a species of *Sanguinicola* from a Nile silurid, but did not name it. Odhner (1924) redescribed this species and named it *S. chalmersi. S. intermedia* was described by Ejsmont (1926) from European cyprinids. He also presented information on *S. armata, S. inermis* and *S. chalmersi* as well as additional life cycle data. Van Cleave and Mueller (1932) described *S. occidentalis* from a percid fish, the walleye (*Stizostedion vitreum*), in Oneida Lake, New York. More information on the biology and ecology of this species was presented by Van Cleave and Mueller (1934). Fischthal (1947) found *S. occidentalis* fairly widely distributed in the walleyes of northwest Wisconsin, and also reported this species from an additional percid host, the yellow perch (*Perca flavescens*).

The present species was first observed April 15, 1944, when six adults were recovered from one of 11 *Huro salmoides* collected from Bear Lake, Barron County. On September 20, 1944, seven of 12 *Huro salmoides* from Lost Land Lake, Sawyer County, were found parasitized, two with one worm each and five with two each. Also on this date, seven worms were recovered from one of two *Micropterus d. dolomieu* from Lost Land Lake. On October 4, 1945, a fingerling *Huro salmoides* from Spring Creek, Washburn County, was infected with one worm. The author recalls finding in 1940 a single, typically triangular *Sanguinicola* egg in the ureter of *Huro salmoides* from the Huron River, Washtenaw County, Michigan. In all probability it belonged to the species herein described.

The name *Sanguinicola huronis* is now proposed for this species. Its description is based on whole mounts of specimens fixed in AFA, stained in Ehrlich's acid haematoxylin, and mounted in balsam. The interpretation of the morphology presented many difficulties as encountered by other workers on the genus *Sanguinicola.*

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[1] Contribution No. 1 from the Department of Biology, Triple Cities College of Syracuse University, Endicott, New York.

Unfortunately, the scarcity of good specimens and living material for study prevented an exhaustive morphological study.

Sanguinicola huronis n. sp.

Description: *Sanguinicola.* Body small, delicate, tapering slightly at both ends; caudal appendage absent; presence of cuticular spines not determined. Mouth anterior; no muscular specialization of oral region; pharynx absent; esophagus slender, terminating in four short intestinal caeca at one-fourth body length from anterior end.

Ovary only slightly H-shaped, occupying nearly entire width of fifth one-sixth of the body length. Oviduct thin-walled, arising from dorsum of ovary, making a short loop over latter, passing posteriad nearly to posterior end of body, then turning anteriad into oötype. Oötype thin-walled, bulbous, dorsal to distal end of oviduct. Uterus short, thin-walled, continuing from oötype, first anterolaterad, then laterad, to female genital pore located dorsally between midline and right margin of body. Limits of vitellaria not discernible. Single, thin-walled vitelline duct first visible posterior to ovary coming from left side, crossing oviduct dorsally, then proceeding posteriad ventral to uterus and oötype, finally uniting with oviduct in turn of latter before oötype. Testes very indistinct, lying between intestinal caeca and ovary; number of follicles not determined. Vas deferens thin-walled, passing posteriad from testes dorsal to ovary and beginning of oviduct; vas deferens continues posteriad near mid-line with only slight undulations before turning postero-laterad and crossing uterus dorsally to male genital pore situated dorsally between midline and right margin of body. Female and male genital pores arranged in tandem, with latter pore posterior; pores opening to outside separately, not into common atrium.

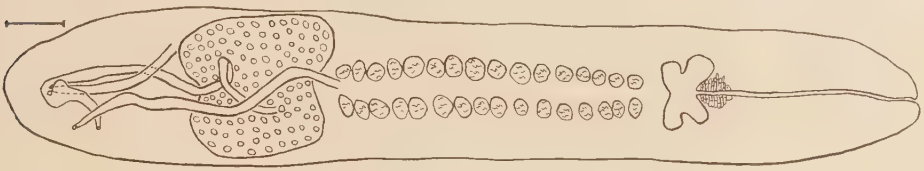

FIG. 1. *Sanguinicola huronis,* adult, dorsal view. The value of the scale is 0.05 mm.

Mean measurements in millimeters (with minima and maxima in parentheses) of seven adult worms for body length and width, and only four of these for the other dimensions are: body length 0.794 (0.746–0.845), width 0.127 (0.076–0.145); ovary length 0.132 (0.105–0.178); esophagus length 0.187 (0.172–0.205); posterior margin of ovary to posterior end of body, 0.148 (0.112–0.205); female genital pore to posterior end of body, 0.076 (0.064–0.088); male genital pore to posterior end of body, 0.061 (0.054–0.063); posterior margin of oötype to posterior end of body, 0.03 (0.026–0.036).

Hosts and localities: *Huro salmoides* from Bear Lake (Barron County), Lost Land Lake (Sawyer County), and Spring Creek (Washburn County); *Micropterus d. dolomieu* from Lost Land Lake (Sawyer County); all in Wisconsin, U. S. A.

Habitat: Mesenteric blood vessels.

Type: U. S. Nat. Mus. Helm. Coll. No. 37111 (from *Huro salmoides,* Lost Land Lake).

S. huronis differs from all known species of *Sanguinicola* in that its definitive host is a centrarchid fish; also, in having the female and male genital pores arranged in tandem. It further differs from *S. inermis* in that its oviduct and vas deferens are not nearly as sinuous, the ovary is only slightly H-shaped, the distance from the posterior margin of the oötype to the posterior end of the body is only one twenty-sixth of the body length (not one seventh), and its esophagus is only slightly more than one half as long. The absence of a rostellum-like anterior end further differentiates *S. huronis* from *S. armata* and *S. intermedia.* It further differs from *S. chalmersi* in that the vas deferens crosses the oviduct dorsally (not ventrally) and has only very slight undulations, the oviduct makes a single loop over the ovary and not far posterior to it, and the vitelline duct enters the oviduct in the anteriad turn of the latter before the oötype and not prior to this turn. Further

differentiation from *S. occidentalis* is shown in the lack of a caudal appendage, the possession of only one vitelline duct posterior to the ovary, an esophagus which is only two thirds as long, an ovary which is only slightly H-shaped and approximately one half as long, and having the distance from the posterior margin of the oötype to the posterior end of the body only one third as long.

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GERMINAL DEVELOPMENT IN THE MOTHER SPOROCYST AND REDIA OF *HALIPEGUS ECCENTRICUS* THOMAS, 1939*

D. J. Ameel, W. W. Cort, and Anne Van der Woude

INTRODUCTION

In recent studies of germinal development in rediae (Cort, Ameel, and Van der Woude, 1948) it was found that in a psilostome and several echinostome species well developed germinal masses were present in the posterior end of the body cavity of both mother and daughter rediae. These masses are attached to the wall by strands of tissue and are composed of both unicellular and multicellular components. The unicellular components are interpreted as germinal cells and the multicellular components as very immature embryos that have started their development while still attached to the mass. These germinal masses appear to be persistent centers of multiplication of germinal cells, and the multicellular components which continually break off from them furnish the constant stream of free embryos that develop in the body cavities of the rediae, and escape from them. In mature and sometimes even in old rediae these germinal masses still persist, and free embryos in all stages of development are present, the smallest of which correspond in structure to the largest multicellular components of the germinal mass. Germinal masses of this type provide for the continuous production of embryos throughout the whole life of the rediae, and make possible the production of rather large numbers of individuals. The important thing, then, is not so much the presence in these germinal masses of small embryos (multicellular components), as the ability of some of their germinal cells (unicellular components) to continue dividing so that new embryos can be produced over such a long period of time. This type of germinal mass can be traced back in the development of the redial embryos to a stage in which it is represented only by a morula-like group of germinal cells in the primitive body cavity (Cort, Ameel, and Van der Woude, 1949, figs. 1, 4, 8, 9).

Since the psilostomes and echinostomes belong to the order Fasciolatoidea Szidat, 1936, which we consider to be the most primitive group of the digenetic trematodes, it becomes of special interest to study the germinal development in rediae of more advanced and specialized groups. In the summer of 1947 we had a particularly favorable opportunity to study the germinal development of *Halipegus eccentricus* Thomas, 1939. This species, which belongs to the family Hemiuridae, appears to represent a very highly specialized type. Its miracidia have lost their free life and are very complicated in structure (Thomas, 1939). The mother sporocyst grows to large size and produces large numbers of rediae, which grow into large elongate sacs without locomotor appendages, and with a reduced digestive system. Finally, the cercaria of this species and, as far as known, those of the whole family, have extraordinarily modified tails with a variety of peculiar appendages. The bodies of the cercariae are withdrawn into a chamber at the base of the tail, and

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infection of the intermediate host is by passive ingestion. All these, as well as other characteristics, indicate that the HEMIURIDAE is a very highly specialized trematode family. It was interesting, therefore, to compare the method of multiplication in the germinal sacs, especially in the rediae, of such a highly specialized form as *H. eccentricus* with that of representatives of the order FASCIOLATOIDEA.

Halipegus eccentricus, the life cycle of which was worked out by Thomas (1939), is found in the eustachean tubes of the green frog, *Rana clamitans,* in the Douglas Lake region. Its intermediate hosts are species of *Physa.* There is only one generation of rediae. Its cystophorous cercariae are swallowed by copepods in the body cavity of which they undergo development to the infective stage in from two to three weeks. After infected cyclops are swallowed by tadpoles, the immature flukes remain with little development in the cardiac end of the stomach until the beginning of metamorphosis when they migrate up the esophagus into the mouth cavity.

For our studies we obtained the eggs of *H. eccentricus* by teasing apart mature flukes which were removed from the eustachean tubes of green frogs. Experimental infections were produced by feeding these eggs which contained fully developed miracidia to two species of laboratory raised snails, *Physa gyrina* and *Physa* sp. We also had for comparative study natural infections in three specimens of *Physa* sp. from a pond in Wilderness State Park about 20 miles north of the Biological Station. Adults were also obtained from infected frogs from this pond. As in our earlier studies, all observations were made on living material, usually with oil immersion lenses. For certain of the observations the living specimens were stained with neutral red. With an abundance of material we were able to study a graded series of developmental stages of the germinal sacs and to check our observations repeatedly at different times during the summer. A brief abstract of our results has already been published (Ameel, Cort, and Van der Woude, 1947).

OBSERVATIONS ON GERMINAL DEVELOPMENT

Lots of snails were placed with viable eggs of *H. eccentricus* on July 2, 18, and 29, and on August 6, 1947, but for some unknown reason only the snails exposed on July 2 and 18 became infected. After each lot of snails was exposed, numbers of empty egg shells were found in the fecal pellets indicating that the eggs had hatched. Subsequent examinations revealed that all of the snails of the first two lots were infected, but that the specimens of *P. gyrina* were heavily infected while those of *Physa* sp., a small dark species, were only lightly infected.

The eggs of *H. eccentricus* are relatively large and the shells are sufficiently clear to permit observation of the larger structures of the fully developed miracidia within them. The anterior two-thirds of the miracidium is occupied by a large median and lateral penetration glands. The germinal mass is located in the posterior third and consists of a group of unicellular components arranged in the form of a morula (Fig. 1). These unicellular components are interpreted as being germinal cells. No measurements of newly hatched miracidia were made, but according to Thomas (1939) they have a length of 0.070 to 0.082 mm and a width of about 0.03 mm.

The earliest examination of a snail was made five days after exposure to the embryonated eggs. It was heavily infected, yielding large numbers of young mother sporocysts (Fig. 2). They varied in length from 0.066 to 0.108 mm and in width

from 0.018 to 0.025 mm. They were very mobile and capable of considerable elongation. When the mother sporocysts contracted, the entire body surface became rugose. The penetration glands were not as well defined as they were in the miracidia but both median and lateral glands were present. Two flame cells were located adjacent to the germinal mass, their ducts opening into well defined lateral bladders. The germinal mass had not increased appreciably in size, and all its components were unicellular.

The next examination was made 11 days after infection. The mother sporocysts at this time showed considerable variation in development. In the smaller individuals the germinal material was crowded closely together and consisted of numerous germinal masses composed of unicellular and multicellular components which almost filled the body cavity and were attached to its wall (Fig. 3). In others, that were considerably larger, a number of germinal masses consisting of both unicellular and multicellular components were attached to the wall of the body cavity at various places, but one was always located at the posterior end (Figs. 4 and 5). At this stage a few small embryos were free in the body cavity, the smallest of which were about the same size as the largest multicellular components of the germinal masses. There was considerable variation in the arrangement of germinal material in the developing mother sporocysts. Figure 4 shows one type in which only the germinal mass at the posterior end contained both unicellular and multicellular components while the remainder of the germinal components which

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PLATE I. Stages in the Development of the Mother Sporocyst of *Halipegus eccentricus*.

Description of Figures

FIG. 1. Diagrammatic drawing of miracidium inside of egg showing germinal mass composed of a morula-like group of unicelluar components.

FIG. 2. Very young mother sporocyst, 0.067 mm in length, from snail examined 5 days after first exposure to eggs, showing germinal mass still as a group of unicellular components.

FIG. 3. Very young mother sporocyst, 0.095 by 0.030 mm, from snail that had been experimentally infected eleven days before; germinal masses almost fill the body cavity and are attached to its wall.

FIG. 4. Young mother sporocyst, 0.160 by 0.084 mm, from snail that had been first exposed to infection 11 days before; germinal mass at posterior end composed of unicellular and multicellular components, and masses of multicellular components attached to wall and forming strings across the body cavity.

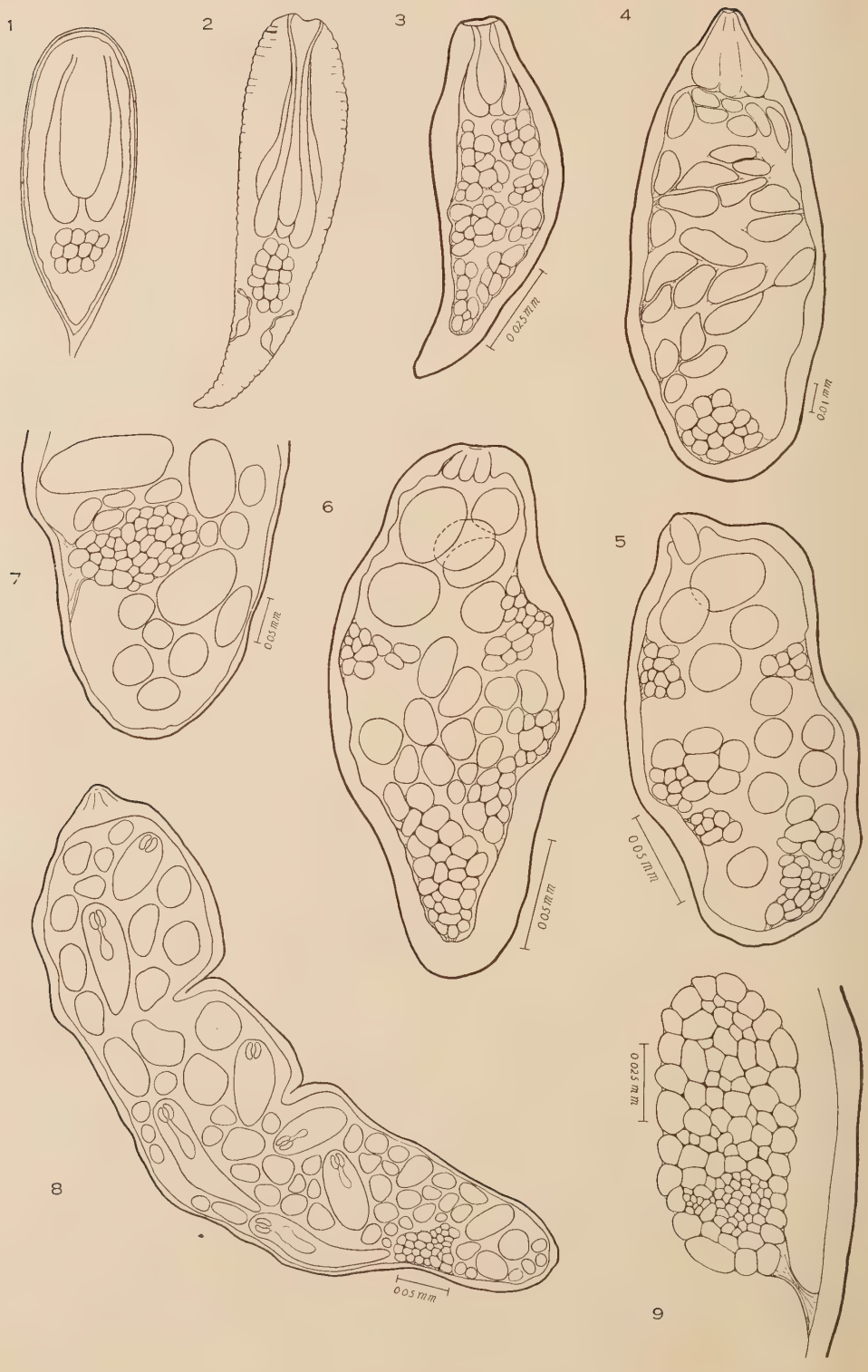
FIG. 5. Young mother sporocyst, 0.22 by 0.11 mm, from snail that had been first exposed to infection 11 days before; germinal masses composed of both unicellular and multicellular components attached to wall at various places, with a well defined mass at the posterior end; the largest free embryo measures 0.040 mm by 0.02 mm.

FIG. 6. Young mother sporocyst, 0.26 by 0.09 mm, from snail that had been first exposed to experimental infection 11 days before; germinal masses composed of both unicellular and multicellular components attached to wall of body cavity at various places; mass at posterior end large and very clearly defined; a number of free embryos present, the largest measuring about 0.036 by 0.030 mm.

FIG. 7. Posterior end of immature mother sporocyst, 0.84 by 0.30 mm, from snail that had been exposed to experimental infection 13 days before; only one large germinal mass present near the posterior end, composed of both unicellular and multicellular components.

FIG. 8. Rather small mature mother sporocyst, 0.70 by 0.15 mm, from snail first exposed to experimental infection 24 days before; a single large germinal mass near posterior end; numerous embryos in the body cavity, the largest of which appear to be ready to escape.

FIG. 9. Germinal mass from old mother sporocyst from snail first exposed to experimental infection 39 days before; composed of numerous components both unicellular and multicellular.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were all multicellular were attached to the wall and extended across the body cavity in the form of strings.

A series of nine mother sporocysts recovered 13 days after infection ranged in size from 0.15 by 0.07 mm to 0.84 by 0.30 mm. In one individual measuring 0.542 by 0.252 mm, one redial embryo, measuring 0.080 by 0.048 mm, with a pharynx was observed. In addition there were 93 embryos of various sizes. Another mother sporocyst, measuring 0.54 by 0.18 mm contained 72 embryos. The largest individual of this series contained two redial embryos in which the pharynx could be distinguished, the largest of which measured 0.10 by 0.06 mm. There was a single large germinal mass near the posterior end of this mother sporocyst (Fig. 7). In the older mother sporocysts all but the posterior germinal mass had been dissipated in the formation of embryos. It was located either directly at the posterior end or was attached at one side.

Some mother sporocysts examined 24 and 25 days after infection contained redial embryos which appeared to be well developed and ready to escape (Fig. 8), but no rediae were found in the tissues of the snails until the twenty-sixth day. Even at this time numbers of mother sporocysts contained nothing but immature embryos, and some large mother sporocysts recovered 31 days after infection contained no mature redial embryos. Counts of the redial embryos in 4 mother sporocysts about 1 mm in length were 95, 100, 101 and 109 respectively.

Mother sporocysts were recovered from experimentally infected snails until the end of the experiment, 39 days after infection. In all instances, though some of them were approaching exhaustion, large germinal masses were observed attached at their posterior ends consisting of both unicellular and multicellular components (Fig. 9). Only a few embryos remained in some of these mother sporocysts, but the smallest were always about the size of the multicellular components of the germinal mass.

During the course of the examinations, it was observed that there was considerable variation in the sizes and stages of development of the mother sporocysts found in individual snails. Since the snails were transferred from the dishes containing the eggs to aquaria within 24 hours after exposure, there was no likelihood of miracidia invading them after the first infection. No actual count was made of the mother sporocysts recovered from the individual snails, but frequently the numbers were such as to indicate the possibility of overcrowding affecting their normal rate of development.

There is but one redial generation in *H. eccentricus*. The development of the germinal material of the redia closely parallels that of the mother sporocyst. In young redial embryos in which the pharynx was just barely discernible, the germinal mass consisted of a morula-like mass of unicellular components like that of the miracidium (cf. Figs. 1 and 10). As the body of the redial embryo elongated, the germinal mass elongated and continued to fill most of the body activity. All the components of the germinal masses in redial embryos less than 0.10 mm in length seemed to be unicellular (Fig. 11). Early in development an irregular cavity appeared in the center of the germinal mass. As development progressed this cavity enlarged and the initial germinal mass broke up into a number of individual germinal masses which adhered to the body wall (Fig. 12).

Redial embryos measuring around 0.23 mm in length seemed to be ready to escape from the mother sporocyst. At this stage of development, the germinal material was variously organized. In some individuals a number of germinal masses were attached to the body wall in linear fashion extending from the posterior end of the gut. Most of their components were unicellular but some appeared to be multicellular (Fig. 12). In other rediae in which the germinal masses were similarly organized, there were considerable numbers of multicellular components (Fig. 13). Many redial embryos, similar to the above were observed, in which in addition the unicellular and multicellular components extended in strands across the body cavity, and a few small free embryos were present (Fig. 14). All of the above characteristics, likewise, were observed in recently emerged rediae. As development proceeded after escape from the mother sporocyst, increasing numbers of free embryos appeared in the body cavity while the lateral germinal masses and strands of multicellular components gradually decreased in size. This is shown in a young redia from a natural infection (Fig. 15). This redia measured 0.48 mm in length and contained 39 free embryos most of which were not more than twice the size of the largest multicellular components of the germinal masses. Older rediae became packed with free embryos and nothing remained of the germinal material except a large germinal mass attached at or near the posterior end (Figs. 16 and 17). In even the larger young redia there was a striking uniformity in the size of the embryos. This is probably due primarily to the rapid early development of large numbers of embryos in connection with early expansion of the germinal mass, but a lag in development of the embryos is also a possibility. In the older rediae filled with large numbers of embryos, there was a noticeable reduction in the size of the gut.

PLATE II. Rediae of *H. eccentricus* in Different Stages of Development.

Description of Figures

FIG. 10. Redial embryo, 0.08 by 0.046 mm, from an experimental infection, showing germinal mass composed of a morula-like group of unicellular components (germinal cells).

FIG. 11. Redial embryo, 0.092 mm long, from an experimental infection, showing germinal masses consisting only of unicellular components attached to wall of body cavity and filling most of its space.

FIG. 12. Redial embryo, 0.220 by 0.044 mm, from an experimental infection, showing germinal masses attached to wall of body cavity; most of the components are still unicellular, but some appear to be muticelluar.

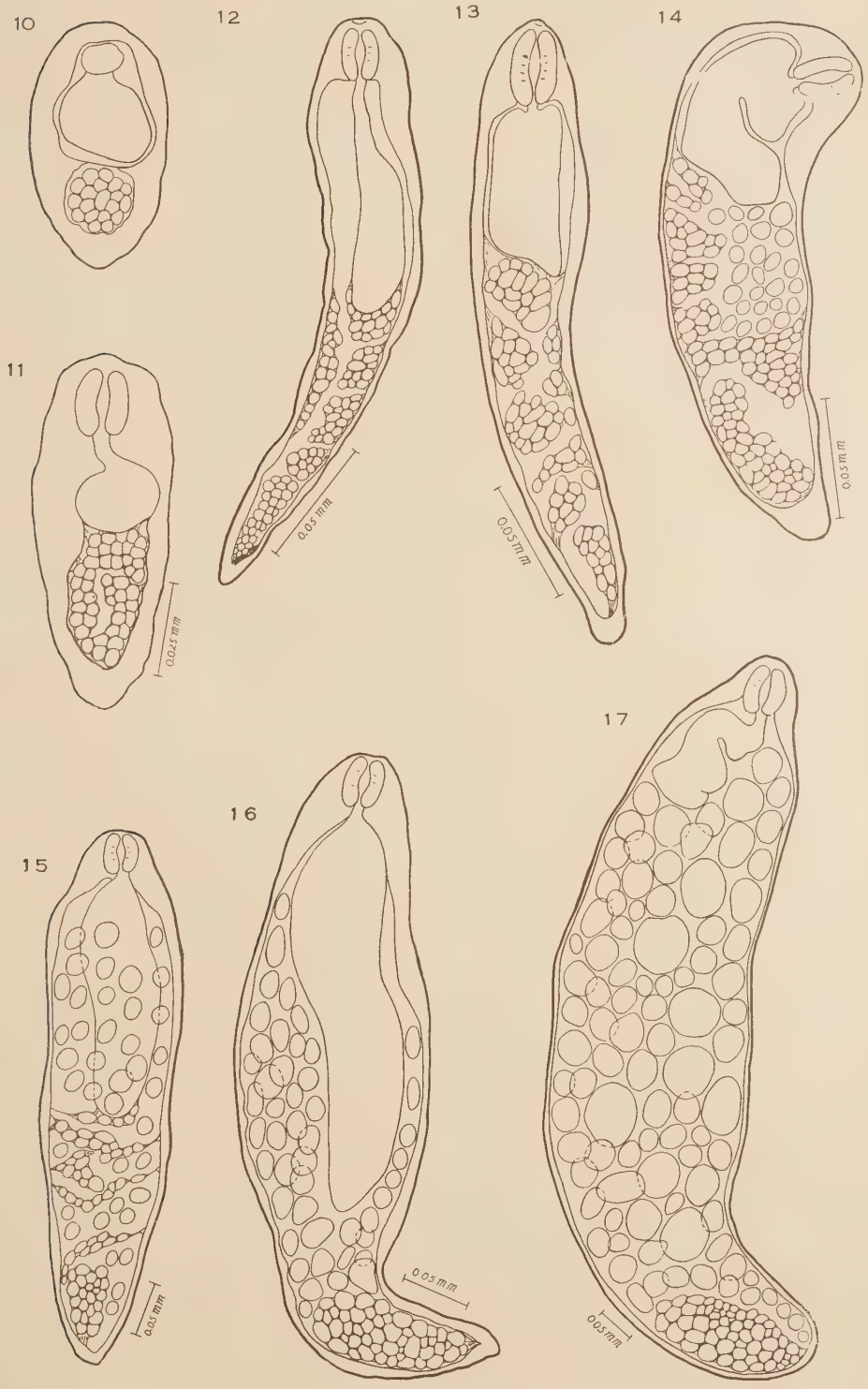
FIG. 13. Redial embryo, 0.24 by 0.05 mm, from an experimental infection, showing germinal masses attached at various places along the wall of the body cavity; a considerable proportion of the components are clearly multicellular, but there are no free embryos.

FIG. 14. Very young redia, 0.230 by 0.088 mm, from an experimental infection, showing germinal masses along wall of body cavity and along strands across the cavity; a number of small embryos are free in the body cavity.

FIG. 15. Young redia, 0.48 by 0.12 mm, from a natural infection, showing germinal masses attached to wall of body cavity and in strands across it; a considerable number of small embryos are free in the body cavity, and there is a definite germinal mass in the posterior end.

FIG. 16. Young redia, 0.50 by 0.16 mm, from a natural infection showing a large germinal mass at the posterior end with both unicellular and multicellular components; large numbers of free embryos are present in the body cavity.

FIG. 17. Young redia, 0.98 by 0.15 mm, from natural infection, showing a single large germinal mass in the posterior end containing both unicellular and multicellular components; large numbers of embryos of variable sizes are present in the body cavity.


10
11
0.025 mm
12
0.05 mm
13
0.05 mm
14
0.05 mm
15
0.05 mm
16
0.05 mm
17
0.05 mm

In the oldest experimental infection studied, 39 days after exposure to the miracidia, a few of the embryos in some of the largest rediae had assumed the characteristic cercarial form.

It was not possible to determine the number of rediae produced by individual mother sporocysts in the laboratory infected snails because of the prevalence of multiple infections. However, in three naturally infected snails, the redial count was 71, 107, and 147. Considering the rapid early development of the germinal material in young mother sporocysts and the large size of their germinal masses it seems evident that the number of rediae that would develop in an infected snail would be determined by the available space and food supply rather than by the reproductive potential of the mother sporocyst. The largest of these rediae contained great numbers of fully developed cercariae and embryos. One hundred and seventy-two embryos were counted in a redia measuring 0.50 by 0.15 mm; another redia, measuring 0.85 by 0.22 mm, contained 342 embryos; and a redia, measuring 2.20 by 0.30 mm, contained 419. Large germinal masses were observed in all of these rediae. The presence of numerous small embryos as well as large numbers of embryos in various stages of development indicated that the peak of productivity had not been reached in any of the natural infections studied. The great productivity of this species of trematode is apparent. A conservative estimate based on the above data would indicate the possibility of the presence of as many as 30,000 to 40,000 cercarial embryos in a single snail at the time of examination. The number of cercariae produced during the productive period of these rediae would of course be many times this number.

DISCUSSION

It is interesting to compare the germinal development in *Halipegus* with that of representatives of the FASCIOLATOIDEA. The first and perhaps the most striking difference is that the mother sporocyst of *Halipegus* is a very large sac in which a complicated germinal development, which is strikingly similar to that of the redia, gives rise to a large number of embryos. We have had as yet no opportunity to study the germinal development in the mother sporocysts of any of the FASCIOLATOIDEA. As far as known, however, they are small sacs that produce only a small number of rediae; therefore, germinal development in them would be expected to be very simple. The germinal development in the rediae of *Halipegus* is distinctly more complicated than that of the rediae of any of FASCIOLATOIDEA studied. In spite of this, very immature redial embryos show a simple germinal mass that consists of a morula-like group of germinal cells very much like that of very small embryos of psilostomes and echinostomes (cf. fig. 10 with figs. 1, 4, 8, 9 in Cort, Ameel and Van der Woude, 1949). The chief difference in the further development in *Halipegus* is a very rapid early multiplication of germinal cells which causes the germinal mass to extend throughout the body cavity; later it breaks up into a complicated series of germinal masses attached to the walls of the greatly extended body cavity (Figs. 12 to 14). Soon after the embryos begin to break away it appears that all division of germinal cells is completed except in the single germinal mass attached at the posterior end of the body cavity, and later it only is left as a

very large complicated structure in which division of germinal cells appears to continue throughout the life of the infection (Fig. 16). This rapid early division and extension of the germinal mass, and possibly a lag in the development of free embryos, produces large numbers of cercarial embryos in the larger rediae many of which are at about the same stage of development. While the mature redia of *Halipegus* resembles that of a psilostome or an echinostome in having a single germinal mass attached at the posterior tip of the body cavity, this mass is much larger and has many more components than any found in rediae of the FASCIOLATIODEA (cf. figs. 16 and 17 and figs. 33, 34, 39, 40 of Cort, Ameel, and Van der Woude, 1948). Therefore, in *Halipegus,* a representative of a very highly specialized trematode family, germinal development while retaining the same general pattern in the rediae has become considerably more complex than in the FASCIOLATOIDEA, and is able to produce much larger numbers of individuals.

SUMMARY

In the summer of 1947 studies were made of the germinal development in mother sporocysts and rediae of *Halipegus eccentricus* Thomas, 1939, in experimentally infected snails of the genus *Physa.* In the miracidium and early mother sporocyst of this species there is a morula-like germinal mass, located at the posterior end, consisting of unicellular components regarded as germinal cells. As the mother sporocyst elongates, this mass of germinal cells increases in size and breaks up into a number of individual masses consisting of both unicellular and multicellular components attached to and distributed along the body wall. All the masses may remain compact or some may take the form of strings extending across the body cavity. By the time the mother sporocysts reach maturity, only a single posteriorly located germinal mass is present, and free embryos fill the body cavity. Germinal masses of very large size and complexity were still present in mother sporocysts 39 days after infection. Within 26 days some redial embryos had escaped from the mother sporocyst.

H. eccentricus has but one generation of rediae. The course of development and organization of the germinal material in the redia is similar to that of the mother sporocyst. In very young redial embryos there is a morula-like mass of germinal cells in the primitive body cavity just back of the developing intestine. As the redial embryos elongate the body cavity becomes larger and is practically filled with the rapidly growing germinal material. In older embryos and young free rediae, numerous germinal masses are present all along the walls of the body cavity, or in some cases they may form strings extending across it. Later, all of these break up into free embryos except a single large germinal mass attached at the posterior end which is still present and giving off embryos in mature rediae. The very rapid early multiplication of the germinal material, and the unusually large germinal masses provide for the production of large numbers of individuals. It was estimated that in natural infections as many as 30,000 to 40,000 cercarial embryos were present at the time of examination, and that the total cercarial production in such infections would be many times that number.

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GERMINAL MASSES IN REDIAL EMBRYOS OF AN ECHINOSTOME AND A PSILOSTOME*

W. W. Cort, D. J. Ameel, and Anne Van der Woude

INTRODUCTION

In a recent paper (Cort, Ameel, and Van der Woude, 1948) we reported work done during the summer of 1947 on the germinal material in rediae belonging to the order Fasciolatoidea Szidat, 1936. In mother and daughter rediae of different ages of a psilostome and several echinostome species we found rather large germinal masses, composed of both unicellular and muticellular components, which were attached at the posterior end of the body cavity. They were interpreted as persistent centers of multiplication of germinal cells (unicellular components), in which some of the elements had developed into small embryos (multicellular components). The breaking off of the multicellular components throughout the reproductive life of the rediae is considered to be the mechanism for the production of the constant stream of free embryos which develop in the body cavities of the rediae and escape from their birth pores. These germinal masses were found in very immature rediae in some of which there were very few free embryos or none at all (l. c. Pl. II, fig. 20, Pl. III, fig. 25). In all these young rediae the germinal masses, with one possible exception (l. c. Pl. IV, fig. 41), appeared to be composed of both unicellular and multicellular components. However, we made the rather obvious suggestion that at earlier stages in very small redial embryos the germinal masses would be composed only of unicellular components. It seemed worthwhile, therefore, when we had an opportunity, to trace back the development of germinal masses of this type into very small redial embryos.

In the summer of 1948 we found a very immature infection of an echinostome species, which we were unable to identify, in a specimen of *Helisoma campanulatum smithii* (Baker). It consisted of a mother sporocyst containing several mother redial embryos of varying sizes and a few free mother rediae in different stages of development. Soon afterward we found two infections of *Psilostomum ondatrae* (Price, 1931) in which only mother rediae were present, one in *H. antrosum percarinatum* (Walker) and the other in *H. campanulatum smithii*. From this material we were able to study very small embryos of both mother and daughter rediae of these two species. All these studies were made on living material examined with oil immersion lenses. We found *intra vitam* staining with dilute neutral red solutions very helpful in the differentiation of the germinal masses in the small redial embryos.

RESULTS

The Echinostome: The smallest mother redial embryo which we were able to study from the echinostome mother sporocyst was only 0.083 mm in length. Its

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Description of Figures

Figs. 1–7 are from an infection of an unidentified echinostome species found in *Helisoma campanulatum smithii,* consisting of a mother sporocyst and several free mother rediae of different sizes.

Fig. 1. Very immature mother redial embryos, 0.083 by 0.038 mm, taken from mother sporocyst.

Fig. 2. Mother redial embryo, 0.142 by 0.037 mm, taken from mother sporocyst.

Fig. 3. Mature mother redia, 0.255 by 0.105 mm.

Fig. 4. Very immature daughter redial embryo, 0.08 by 0.04 mm, taken from a mother redia.

Fig. 5. Daughter redial embryo, 0.173 by 0.058 mm, taken from a mother redia.

Fig. 6. Daughter redial embryo 0.180 by 0.045 mm, taken from a mother redia.

Fig. 7. Young daughter redia, 0.195 by 0.045 mm, after escape from the mother redia.

Fig. 8–12 are from two infections of *Psilostomum ondatrae* from *H. campanulatum smithii* and *H. antrosum percarinatum.*

Fig. 8. Very immature daughter redial embryo, 0.07 by 0.05 mm, taken from a mother redia.

Fig. 9. Daughter redial embryo, 0.192 by 0.065 mm, taken from a mother redia.

Fig. 10. Daughter redial embryo, 0.18 by 0.04 mm, taken from a mother redia.

Fig. 11. Daughter redial embryo, 0.218 by 0.064 mm, taken from a mother redia.

Fig. 12. Young mother redia, 0.243 by 0.038 mm, found free in the digestive gland of the snail host.


1
0.025 mm
2
0.025 mm
3
0.05 mm
4
0.025 mm
5
0.025 mm
6
0.025 mm
7
0.025 mm
8
0.025 mm
9
0.05 mm
10
0.05 mm
11
0.05 mm
12
0.05 mm

germinal mass consisted of a morula-like group of germinal cells in the primitive body cavity (Fig. 1). In a somewhat larger embryo also taken from the mother sporocyst the body cavity had extended forward and there were present several free embryos in front of the germinal mass, which was attached at the posterior end of the body cavity and which also consisted only of unicellular components (Fig. 2). A very immature daughter redial embryo, 0.08 by 0.04 mm, taken from one of the mother rediae also had a germinal mass in its primitive body cavity containing only unicellular components (Fig. 4). In somewhat larger daughter redial embryos with a few free cercarial embryos in their body cavities, the germinal masses still clearly contained only unicellular components (fig. 5 and 6), but, in a somewhat larger daughter redia which had already escaped from the mother, a few multicellular components could be made out at the anterior end of the germinal mass (fig. 7).

Psilostomum ondatrae: In a series of daughter redial embryos of *P. ondatrae* taken from mother rediae, the germinal masses also contained only unicellular components. The youngest of these was the smallest redial embryo (0.07 by 0.05 mm) in which the germinal mass could be clearly made out (Fig. 8). Another embryo, more than twice the length of the smallest had a similar germinal mass still in a primitive body cavity (Fig. 9). Figures 10 and 11 also show germinal masses consisting only of unicellular components in much larger daughter redial embryos. Figure 12, the only mother redia of this series, also shows only unicellular components in its germinal mass. Figures 11 and 12 are interesting in showing changes in shape of the germinal cells in the masses due to contraction and elongation of the rediae.

SUMMARY

These studies on embryos of rediae of the suborder ECHINOSTOMATA Szidat, 1939, show that in very early stages the germinal mass consists only of unicellular components, and it is only in later development, after some free embryos are present, that some of the germinal cells form embryos which remain attached for a while to the mass as multicellular components. It can be suggested that at a much earlier stage of the development of the redial embryos a single cell of the germinal line comes to lie just back of the cells that form the intestine. This cell then goes through a series of divisions forming a morula-like mass of germinal cells in the primitive body cavity which later becomes a typical germinal mass with both unicellular and multicellular components.

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AN ATTEMPT TO CLARIFY THE STATUS OF THE SPECIES IN THE GENUS *OPHIONYSSUS* MÉGNIN (ACARINA: MACRONYSSIDAE)[1]

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In a recent paper (Camin, 1948), the writer suggested that there is only one valid, described species in the genus *Ophionyssus* Mégnin and data are herein presented to support this view.

HISTORICAL REVIEW

The snake mite was first reported by Metaxa (1823) from specimens taken from snakes in a captive collection in Florence, Italy. Dugés (1834) discovered the mite in France and suggested its relationship to a bird mite, *Dermanyssus avium.* In 1844 Gervais described and named the snake mite *Dermanyssus natricis.* Mégnin (1884) later redescribed this mite and erected the genus *Ophionyssus,* but this generic name was not generally accepted because he apparently mistook the narrow, elongate genito-ventral shield for the genital aperture, but he pointed out that the chelicerae were shear-like rather than stylet-like, as in other *Dermanyssidae*; and that the dorsal shield was small and did not cover the greater part of the dorsum, as in most of the closely related genera.

Hirst (1915) discovered some mites on an American "Couper's snake", probably *Drymarchon corais couperi,* at the London Zoological Gardens and, on the basis of five adult female specimens, described a new species, *Ichoronyssus serpentium,* which he admitted might be identical with *Ophionyssus natricis* (Gervais). Under the name *Liponyssus natricis,* Berlese (1918) redescribed a snake mite, which he believed to be the same species originally described by Gervais. He stated that the only difference between his mite and that of Hirst's was that the former had only a single dorsal shield while the latter, according to Hirst's description, had two dorsal shields. Hirst and Berlese both pointed out Mégnin's error, but neither accepted his genus *Ophionyssus.*

In a later paper, Hirst (1921a) described as new another species of reptile mite, *Liponyssus arabicus,* using a single female specimen from a lizard, *Agama adramitana* Anderson. That same year, Hirst (1921b) reported that he had examined specimens labeled *L. natricis,* which he had received from Berlese,[3] and he stated that Berlese had overlooked the minute posterior dorsal shield which these mites had. After examining these specimens, Hirst stated that *L. arabicus* was identical with *L. natricis,* but he still maintained that *L. natricis* and *I. serpentium,* which he

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[3] The mites which Hirst received from Berlese are now in the British Museum collection, but correspondence with Dr. Charles D. Radford has revealed that these slides have deteriorated with age and measurements for purposes of comparison were impossible.

now called *Liponyssus serpentium,* were distinct in that the distance between the dorsal shields of the former was "small" and the dorsal shields of the latter were "widely" separated.

Ewing (1922) erected a new genus, *Serpenticola,* for the snake mite and designated *Liponyssus serpentium* (Hirst) as the type species of the genus. In 1925 Ewing described a second species in this genus as *Serpenticola easti,* on the basis of five female specimens taken from a lizard, *Sceloporus graciosus.* Later, Ewing (1929) established the validity of the genus *Ophionyssus* Mégnin on the basis of the minute posterior dorsal shield of the female and, at the same time, reduced his genus *Serpenticola* to synonymy.

After examining a cotype of *Ophionyssus natricis* (Gervais) and several specimens of snake mites from France and Italy and comparing these with the figures published by Hirst for *Ichoronyssus serpentium* and by Mégnin for *Ophionyssus natricis* (Gervais), André (1937) concluded that the two species were identical. In this same paper, André stated that Berlese (1918) was in error when he identified a snake mite as *Liponyssus natricis* (Gervais) and, furthermore, claimed that it was actually another species. When Hirst (1921b) reported that his *L. arabicus* was identical with *L. natricis* of Berlese, he placed the former into synonymy with the latter. Therefore, André suggested that the name *Liponyssus arabicus* should be revived and used for L. *natricis* of Berlese because *natricis* had been used earlier by Gervais.

Fonseca (1948), in an excellent monograph on the *Macronyssidae,* listed two species, *Ophionyssus natricis* (Gervais) and *Ophionyssus easti* (Ewing), in the genus *Ophionyssus* Mégnin and placed *Liponyssus arabicus* Hirst in the genus *Steatonyssus* Kolenati, calling it *Steatonyssus arabicus* (Hirst). This represents the present status of the aforementioned species, *Ophionyssus serpentium* (Hirst) having been placed into synonymy with *Ophionyssus natricis* (Gervais) by André (1937).

Consideration of *Steatonyssus arabicus* (Hirst)

In his diagnosis of the genus *Steatonyssus* Kolenati, Fonseca (1948) listed a divided dorsal shield in the female, with a large podosomatal shield and a well-developed, elongate opisthosomatal shield; a sternal shield with three pairs of setae and two pairs of pores; and an undivided holoventral shield in the male as characteristic of the genus. For the genus *Ophionyssus* Mégnin, he stated that a divided dorsal shield in the female, with a large podosomatal and a minute opisthosomatal shield; a sternal shield with only two pairs of setae; and a divided holoventral shield in the male, with a sterni-metasterni-genital shield and an isolated anal shield are diagnostic.

Hirst (1921a), in his description of *Liponyssus arabicus,* clearly stated that the posterior dorsal shield of the female was very minute and oval as in *L. serpentium* and that the sternal shield possessed only two pairs of setae. In his later paper of the same year, after having examined specimens of Berlese's *Liponyssus natricis* and concluding that they were identical with his *L. arabicus,* Hirst reiterated that the posterior dorsal shield was very minute.

Examination of the figures published by Berlese (1918) for his *Liponyssus natricis* shows that the egg, larva, nymph, male, and venter of the female are apparently identical with those observed in *Ophionyssus natricis* (Gervais). The

sternal shield of the female bears only two pairs of setae and the holoventral shield of the male is divided into a sterni-metasterni-genital shield and an isolated, pear-shaped anal shield.

The writer believes that these descriptions by Hirst and Berlese show conclusively that this mite is a member of the genus *Ophionyssus* Mégnin and not of *Steatonyssus* Kolenati.

In his description of *Liponyssus natricis,* Berlese (1918) included measurements of several of the shields and the body dimensions of the various stages in the life cycle of the mite, while Hirst, unfortunately, included only the body lengths of *L. serpentium* and *L. arabicus* and the dimensions of the anterior dorsal shield of the former. These measurements are compared with those made by the writer (Table 1). It may be observed that all of the measurements by Berlese and Hirst compare favorably with those made by the writer, except for Berlese's data on the anal shield of the female and on the body lengths of the larva and of the male.

In clearing snake mites, prior to mounting on slides, it has been observed that the area from the posterior part of the anterior dorsal shield to the anterior half of the posterior dorsal shield is the last to clear. Thus, in an incompletely cleared mite, this area plus the area on the ventral surface, covering the anterior half of the anal shield and the posterior part of the genito-ventral shield are poorly defined. The writer believes that this may account for Berlese's failure to observe both dorsal shields and indicates that his data on the anal shield, genito-ventral shield, and dorsal shields may be inaccurate. This is further supported by the fact that Berlese shows no setae on the genito-ventral shield, while at least one pair of setae is present on this shield in all genera of the *Macronyssidae*; and he shows the anal shield as elongate and oval, while Hirst (1921a) clearly stated that the anal shield of *L. arabicus* was pear-shaped. Berlese, himself, shows this shield as pear-shaped in all the other stages of the life cycle.

The discrepancies between the measurements by the writer and those by Berlese on the body lengths of the larva and of the male may be due to a difference in the parts included in the measurements. The writer's data do not include the capitulum, while this part may have been included by Berlese. This seems to be a valid assumption in that the larva measured by Berlese is longer than the egg. The larval mite is a non-feeding stage of appproximately twenty-four hours duration and does not increase in size after hatching.

Hirst (1921b), in his discussion of the differences between *L. natricis* Berlese (not Gervais) and *L. serpentium,* mentioned that the posterior dorsal shields are similar in shape, but longer in *L. natricis.* In his description of *L. arabicus,* Hirst (1921a) stated that *L. arabicus* and *L. serpentium* were similar, except that the anterior dorsal shield of *L. arabicus* was long and wedge-shaped and separated from the posterior shield by a comparatively short space, while the anterior dorsal shield of *L. serpentium* was lemon-shaped and widely separated from the posterior shield. In a "Key to the females of the species of the genus *Liponyssus* present in the British Museum collection", Hirst (1921b) separated the two species as follows:

3a. Anterior dorsal shield small and separated from the minute posterior shield by a considerable space. ..... *Liponyssus serpentium* (Hirst)

b. Anterior dorsal shield rather long and only separated from the posterior one by a short space. ..... *Liponyssus natricis* (Gervais)

TABLE 1.—*Comparison of measurements on the four species of reptile mites. (Measurements in microns)*[1]

Observer		Camin			Hirst		Baker[2]	Berlese
Species		*O. natricis* (Gervais)			*L. serpentium* (Hirst)	*L. arabicus* Hirst	*O. easti* (Ewing)	*L. natricis* Berlese
	No. of specimens	Min.	Max.	Average				
EGG								
L	17	310.2	376.2	349.0				340
W	17	211.2	283.8	258.5				240
LARVA								
Body								
L	9	290.4	330.0	314.6				450
W	9	211.2	257.4	226.6				230
NYMPH								
Body								
L	56	316.8	633.6	395.2				
W	56	198.0	396.0	252.0				
Ant. dors.								
L	54	172.0	220.5	193.3				200
W	53	171.9	235.2	197.4				200
Post dors.								
L	50	41.2	73.5	61.3				60
W	54	73.5	97.0	83.3				90
MALE								
Body								
L	30	462.0	528.0	495.1				560
W	29	257.4	356.4	295.3				280
S-m-g								
L	29	170.5	214.6	198.4				220
W	29	61.7	95.6	78.9				85
Anal								
L	29	72.0	94.1	83.5				100
W	28	49.9	61.7	56.3				50
FEMALE								
Body								
L	66	567.6	1346.4	883.8	900	730	1142	800–1800
W	65	349.8	957.0	624.1				400–1200
Ant. dors.								
L	42	242.6	346.9	304.1	300		300	
W	36	242.6	320.4	283.8	270		280	
Post dors.								
L	41	29.4	59.4	50.1			47	
W	40	42.6	66.2	54.2			53	
Stern.								
L	42	35.2	51.5	42.8				50
W	40	91.1	125.0	109.2				130
G-v								
L	37	242.6	323.4	278.2				280
W	36	73.5	99.6	87.0				80
Anal								
L	40	102.9	125.0	114.7			113	140
W	39	66.0	77.9	72.9				50
Peritreme								
L	41	117.6	139.7	132.7			147	120

[1] Abbrevations used in table: L = length, W = width, Ant. dors. = anterior dorsal shield, Post. dors. = posterior dorsal shield, S-m-g = sterni-metasterni-genital shield, Anal = anal shield, Stern. = sternal shield, G-v = genito-ventral shield.

[2] Dr. Edward W. Baker of the U. S. National Museum very kindly made these measurements at the request of the writer.

At the present time, after the considerable classification and reclassification that these mites have undergone, 3a, *Liponyssus serpentium* (Hirst), would refer to *Ophionyssus natricis* (Gervais) and 3b, *Liponyssus natricis* (Gervais), would be *Steatonyssus arabicus* (Hirst).

Observations and measurements made by the writer on more than sixty adult female snake mites, which were selected at random from a culture of mites known to be of a single species, show that the posterior dorsal shield is quite variable, measuring at one extreme 29.4×42.6 microns and at the other extreme 59.4×66.2 microns. It is sometimes longer than it is wide and sometimes wider than it is long. The anterior dorsal shield is also variable, being somewhat wedge-shaped in some individuals and lemon-shaped in others, with degrees of variation in between.

Statistical analysis of these measurements reveals that there is no correlation between the dimensions of the sclerotized areas (anterior dorsal, posterior dorsal, sternal, genito-ventral, and anal shields and peritremes) and the length of the body of the mite, while there is very significant correlation, with much less than a 1% probability of chance correlation, between the dimensions of the softer parts (width of body, distance between the dorsal shields, and distance between the genito-ventral and anal shields) and the length of body.

Using this analysis it is clear that as the mite increases in size, due to the engorging of host blood, the sclerotized areas do not change in size, while the distances between sclerotized areas do increase very markedly. Thus, on a small, relatively unengorged mite, the anterior dorsal shield would appear rather long and would be separated from the posterior shield by a relatively short space. On the other hand, on a large, heavily engorged specimen, the anterior dorsal shield would appear rather small and would be separated from the posterior shield by a considerable space. These facts are clearly demonstrated by a comparison of two adult female snake mites, one engorged and the other unengorged (Fig. 1).

A comparison of the drawings of the snake mite by Hirst (1915) and those by Berlese (1918) shows clearly that the mites used by Hirst, for his *I. serpentium,* were much more heavily engorged than those used by Berlese. The writer suggests, therefore, that the separation of the two species, *O. natricis* (Gervais) and *S. arabicus* (Hirst), by Hirst (1921b) probably was made on a comparison of engorged and unengorged individuals of the same species.

Consideration of *Ophionyssus easti* (Ewing)

The description given by Ewing (1925) for *Ophionyssus easti* is very incomplete except for the mapping of setae on the anterior dorsal shield, and states that the only difference between this species and *O. serpentium* (Hirst) is in the shape of the anterior dorsal shield and in the fact that *O. easti* has only eighteen setae on the anterior dorsal shield, while Hirst reported twenty setae for *O. serpentium.*

As has been previously noted, the shape of the dorsal shields is quite variable and the number of setae on the anterior shield will vary from eighteen to twenty-two. The marginal setae, depending upon the extent of sclerotization, will sometimes occur on the shield and sometimes off the shield in the surrounding softer integument.

In correspondence, Dr. Edward W. Baker of the U.S. National Museum, stated that he can find no difference between *O. easti* and what has been determined as *O. serpentium.* Measurements on *O. easti,* which Dr. Baker very kindly made for the writer, are included in the table (Table 1) and all fall within the limits of those by the writer and those by Berlese and Hirst. It is suggested, therefore, that the characters used by Ewing for the separation of *O. easti* are merely normal variations occurring in *O. natricis* (Gervais).

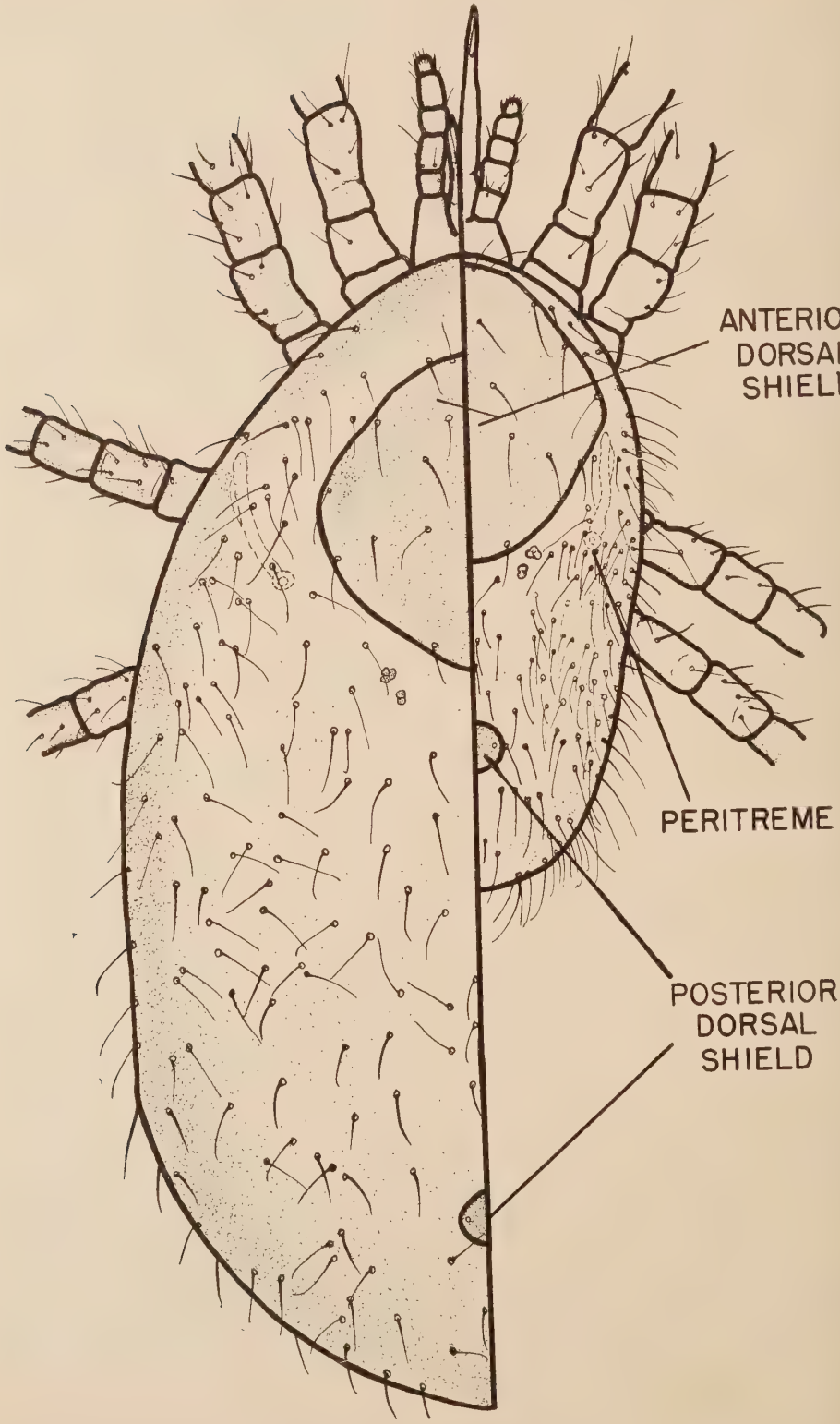

FIG. 1. Comparison of engorged (left) and unengorged (right) snake mites. Drawn to scale with the aid of a projection microscope.

CONCLUSIONS

From the foregoing data, the writer concludes that the four described species of reptile mites discussed in this paper, *Ophionyssus natricis* (Gervais, 1844), *Ophionyssus serpentium* (Hirst, 1915), *Ophionyssus easti* (Ewing, 1925), and *Steatonyssus arabicus* (Hirst, 1921), are in reality a single species and that, by the law of priority, the last three names should be placed into synonymy under *Ophionyssus natricis* (Gervais), which is the only valid described species in the genus *Ophionyssus* Mégnin.

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RESULTS OF EXPOSURE OF THE SNAIL *AUSTRALORBIS GLABRATUS* TO VARYING NUMBERS OF MIRACIDIA OF *SCHISTOSOMA MANSONI*

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It was the purpose of the following experiments to compare infection percentages in the snail, *Australorbis glabratus,* after exposure to definite numbers of miracidia of the trematode *Schistosoma mansoni.* Such data are of value to workers engaged in maintaining colonies of infected snails essential in research in the problems of schistosomiasis and are also the basis of a statistical analysis of the incidence of infection in snails exposed to miracidia. These experiments are a further refinement of previous work done in this laboratory (Schreiber and Schubert, 1949) when it was found that routine exposures of 553 snails to five to seven miracidia of about one to two hours of age resulted in 60 percent of the snails shedding cercariae within four to ten weeks from the time of exposure. The present work describes a comparison of the percentages of snails that shed cercariae between five and ten weeks after exposure to the definite numbers of one, three, seven or twelve miracidia per snail in an effort to extend and make more precise the correlation previously pointed out between the numbers of miracidia used for exposures and the number of snails that eventually discharge cercariae. A serious inadequacy of the work previously reported was that in most of the data presented, no account was given of the snails that died. Thus while it was possible to state the fraction of exposed snails that subsequently shed cercariae, it was not possible to infer that the entire remainder was not infected since the fraction that died had not been recorded.

The miracidia used in all infections were hatched from eggs in fecal pellets obtained from hamsters infected at least eight weeks previously with *S. mansoni* cercariae. About eight fresh fecal pellets were teased apart in 100 ml. Great Bear Spring Water in a finger bowl. After one to two hours at a temperature of 28°–30° C., swimming miracidia were observed under a dissecting microscope. These were drawn up in a droplet of water in a micropipette, and transferred to a 5 ml. beaker. One, three, seven, or twelve miracidia were collected in this manner and then about 3 ml. spring water and a snail 6–8 mm. in diameter were added to the beaker. Although most of the miracidia had disappeared in a few hours, the snails were kept in the 5 ml. beakers overnight at 24–26° C. and transferred to culture aquaria the following day. Four weeks later these snails were individually placed in 5 ml. beakers with spring water and set within eight to twelve inches of a 100 watt electric bulb. They were observed for three to four hours for liberation of cercariae. It has previously been shown that the light and heat under these conditions stimulate the liberation of cercariae from infected snails and that this stimulation is particularly apt to cause liberation of cercariae after intervals of several days during which the snails are not so stimulated. These observations were repeated

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individually on the snails at weekly intervals for the next six weeks or until ten weeks after exposure of the snails to miracidia. Snails were used in routine infections and their subsequent fate is not recorded here. The percentage of snails that died as recorded in Table 1 refers only to snails that had not yet begun to shed cercariae during the period of this experiment. The percentage of snails that died after they began to shed cercariae has been separately studied and reported in the previous work. The figures in Table 1 indicate an increasing percentage of snails

TABLE 1.—*Infection Percentages and Death Percentages In Snails After Exposure to Varying Numbers of Miracidia*

Number of miracidia used for exposures	Total number of snails exposed	5 weeks after exposure to miracidia		6 weeks after exposure to miracidia		10 weeks after-exposure to miracidia		Snails shedding cercariae	Snails surviving 10 weeks but not shedding cercariae	Snails not shedding cercariae and not surviving the 10 week period
		Total number of snails having shed cercariae	Number of snails died	Total number of snails having shed cercariae	Number of snails died	Total number of snails having shed cercariae	Number of snails died	%	%	%
1	121	17	18	17	26	17	50	14	45	41
3	100	0	2	44	4	55	13	55	32	13
7	273	20	14	104	26	192	43	70	15	15
12	112	54	4	91	8	96	10	85	1	14

producing cercariae as a result of exposure to increasing numbers of miracidia. Together with this, there is a decreasing percentage of snails surviving ten weeks but not shedding cercariae. Thus, despite the compulsory close association of snail with as many as seven miracidia, all confined to less than four ml. of water past the

TABLE 2.—*Numbers of Cercariae liberated by Individual Snails, each exposed to 7 Miracidia, Beginning Observations Six Weeks After Exposure*

Date	*Snail* 1	2	3	4	5	6
1946						
5/21	1010	560	810	....	....	....
5/22	600	1072	250	....	....	....
5/23	792	804	990	....	....	....
5/24	574	406	400	336	324	190
5/28	1644	1484	3156	3444	2148	2268
5/29	210	48	252	310	168	264
6/4	880	948	1044	450	288	670
6/5	444	504	112	315	252	340
6/6	624	396	496	594	315	240
6/7	1952	976	688	1072	578	720
6/11	832	1152	1184	1200	1040	176
6/12	2142	3280	3408	1264	656	165
6/13	2512	2226	2080	1624	1472	728
6/14	2816	2682	3760	2052	1152	704
6/17	2480	5472	2016	864	2124	864
6/18	1792	2512	1768	944	1600	672
6/19	1408	1200	1504	288	944	816
6/20	1394	2064	2848	868	944	1078
6/21	540	688	1520	240	364	560
6/25	1134	2196	3078	1572	1464	1956
6/26	208	1264	2196	672	546	1162
6/27	0	522	486	512	160	576
6/28	dead	1312	864	800	1008	1188
7/2	....	1840	1080	680	2472	992
7/3	....	860	210	180	490	230
7/8	....	1100	1270	240	260	200

survival time of the miracidia, large and significant percentages of snails failed to shed cercariae during the next ten weeks.

The justification for assuming snails were not infected on the basis of observations only once a week is that in our experience when infected snails once begin to shed cercariae they can be depended on to liberate some cercariae at least once a day when stimulated. In Table 2 are listed observations on six individual snails, each exposed to seven miracidia and examined individually for cercarial liberation beginning six weeks later. Over the following period of seven weeks during which about twenty four observations were made on each snail, only one observation is recorded in which a snail produced no cercariae and this was the day before its death. On the other hand when infected snails have been liberating cercariae for three to four months it does happen frequently that after this period they may fail to liberate cercariae on stimulation.

SUMMARY AND CONCLUSIONS

1. *Australorbis glabratus* snails were exposed to miracidia of the trematode, *Schistosoma mansoni,* under uniform controlled conditions.

2. Snails exposed to one, three, seven and twelve miracidia demonstrate an increasing percentage of cercariae-producing snails as a result of increasing numbers of miracidia used for exposures.

3. At exposure levels of one to seven miracidia per snail decreasing but still significant percentages of snails fail to shed cercariae ten weeks after exposure.

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PERSISTENCE OF STRAIN-SPECIFIC BEHAVIOR IN TWO STRAINS OF *TRYPANOSOMA CRUZI* AFTER PROLONGED TRANSFER THROUGH INBRED MICE[1]

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Two lethal strains of *Trypanosoma cruzi,* originally isolated from human patients, were carried for over two and three years, respectively, in mice. Opportunity was thereby provided for observations on strain specific virulence, tissue affinity, drug-response, and rhythmic fluctuations with seasonal temperatures. Both strains of *T. cruzi* maintained their characteristic behavior after 63 and 114 serial passages through genetically homogeneous hosts.

MATERIALS AND METHODS

The infection was propagated in male mice of brother-sister mated stock. Homozygosis in these animals was above 98 per cent. Sex of host (Hauschka, 1947) or varying genetic backgrounds were thus eliminated as potential causes of fluctuations in virulence.

The Wellcome-Brazil-Hamburg strain (WBH-strain) of *T. cruzi,* originally derived from a patient in Brazil, was carried exclusively in mice by Dr. E. Reichenow in Hamburg from 1926–1936, by Dr. C. A. Hoare at the Wellcome Laboratories in London since 1936, and in our laboratory since February 1947. This virulent strain at present kills mice within 6–13 days after subcutaneous injection with 0.1 cc of blood taken from the heart on the eighth day of infection. The inoculum averaged about one-half million trypanosomes per mouse. In order to maintain maximum virulence without risking loss of WBH-strain by premature death of all the hosts, two overlapping series of mice have served for passage during the past 28 months. One group of five mice was inoculated every Monday with blood from a mouse infected the preceding Monday, and a similar group every Thursday, with blood from a mouse infected the previous Thursday.

A culture of the less virulent "Brasil" strain (B-strain) was obtained from Mrs. Eleanor Johnson Tobie at the National Institute of Health, Bethesda, Md. The B-strain, derived from a Brazilian patient, had been kept at the National Institute of Health both in vitro and in rats for about six years. Since December, 1945, we have maintained one subline of this strain in culture and another in mice. Survival of mice originally ranged from 11–32 days, but has been narrowed down to from 9–28 days with a mortality of 100 per cent. The method of sub-inoculation was the same as for the WBH-strain, except that transfer took place only every 12 to 14 days at which time the blood population of trypanosomes reached its height.

The mice were kept in groups of not more than five per cage. The Morris-Thompson checker diet (Morris, 1944) was uniform throughout the period under consideration. A mercury thermocouple sensitive to 0.2° F was wired to an electric fan heater in the animal room and was set to prevent a drop below 70° F ± 2° at any

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time. The minimum environmental temperature was thereby established at 68° F, and the maximum reached on rare occasions during prolonged spells of summer heat was 91° F.

Several groups of infected mice totalling 126, were treated for 5–8 consecutive days with the quinoline derivative, Bayer 7602. The daily intraperitoneal injection per mouse was 0.8 mg. of the drug in 0.2 cc of double distilled water. No toxic reactions were observed at this dosage level.

The data for blood populations of *T. cruzi* are based on thick smears of blood obtained by heart puncture on the eighth day of infection with the WBH-strain and on the twelfth day with the B-strain. The smears were made as uniformly thick as possible, were air-dried over night, and were stained and laked simultaneously for ten minutes in a solution of one part Hartmann-Ledden Co. Giemsa stock in twenty parts of distilled water buffered to pH 7.00.

The individual trypanosome counts represent surveys of 100 fields of thick blood smear with an oil-immersion objective at a magnification of 900 diameters. In some of the heaviest infections the parasites were so numerous that only a few representative fields could be counted accurately. For the sake of comparison such counts were then adjusted to 100 fields.

Tissues and organs of infected mice were fixed in Zenker's fluid, paraffin-sectioned six microns thick and stained with Mayer's hemalum and eosin.

RESULTS

1. Virulence

Mortality, plotted in Figure 1, reveals a clearcut and constant difference in virulence between the two strains of *T. cruzi*. All the infected mice died. Curves I and II include 500 mice injected with WBH-strain from February 22nd 1947 to May 31st 1948, and from June 1st 1948 to January 3rd 1949, respectively. No change in virulence reflected in the slope of the survival graph has occurred during the latter period. The same holds true for curves III and IV based on 150 hosts inoculated with B-strain.

Since January 1949, the deaths of an additional 131 mice (not included in Figure 1) showed a lowering of the upper survival limit from 17 down to 13 days for the WBH-strain, and from 32 down to 28 days for the B-strain. The characteristic death rates, however, remained essentially unchanged after 63 B-strain and 114 WBH-strain consecutive passages through a total of 781 mice. It may be concluded that strain-specific maximal virulence had been attained about two years ago and has been maintained since.

2. Tissue Affinity

A similar strain-specificity was apparent in the parasite's affinity for certain host tissues, compared in Table 1. In the case of the B-strain the heart was nearly always riddled with pockets of leishmania stages of the parasite, while only one-fourth of the mice infected with WBH-strain showed light infection of cardiac muscle. The WBH-strain exhibited a definite preference for liver, kidney and spleen, and to a lesser extent for lung. The two strains did not differ much in their invasion of small intestine and skeletal muscle. Gonads and brain were never found infected. Since these histological observations were made on tissues obtained gradually over

a space of about two years, the characteristic tissue affinities of the two strains appear to have persisted quite unchanged.

3. Susceptibility to Bayer 7602

Bayer 7602 (Ac), the acetate of bis (2-methyl-4-aminoquinolyl-6) diallyl malonamide, appears to be the only drug so far used with any degree of success in human cases of South American trypanosomiasis.

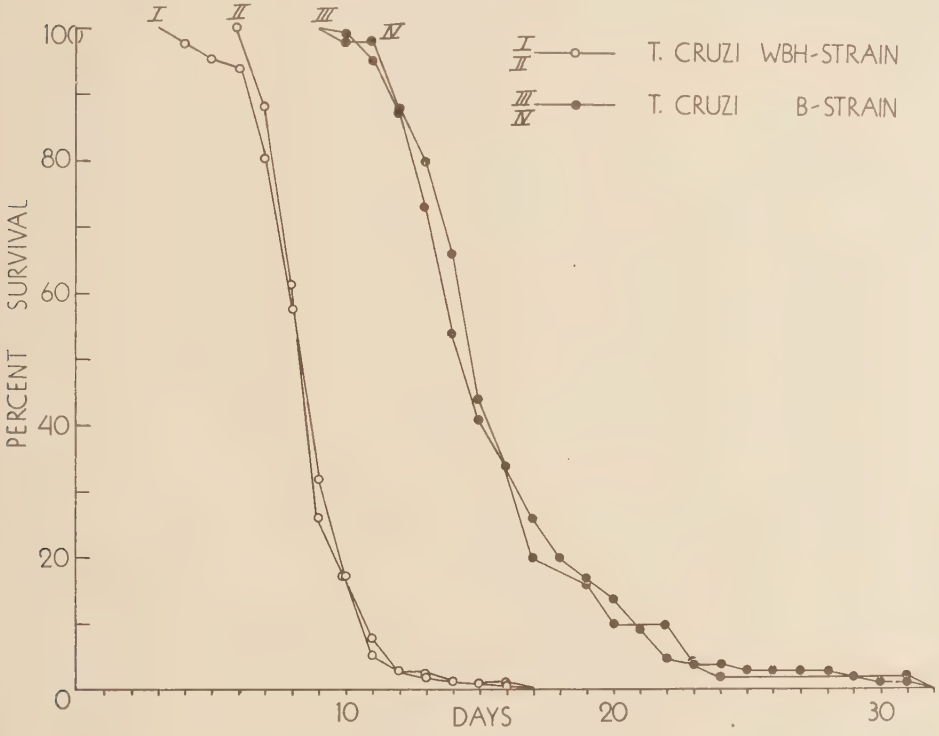

Fig. 1. Strain-specific mortality rate of inbred male mice infected with two lethal strains of *T. cruzi*.

Curve I is based on 400 mice which succumbed to routine serial inoculations of WBH-strain from February 1947 to May 1948. Curve II includes 100 mice infected with WBH-strain between May 1948 and January 1949. Curves III and IV cover the same two arbitrary periods for 150 mice infected with B-strain.

Treatment with daily dosages of 0.8 mg was given for five to seven consecutive days to 99 WBH-infected mice beginning on the second, fourth, fifth and sixth day of infection, respectively. Seventy-six mice (76.8 per cent) in this series recovered while the remaining 23 died of Chagas' disease. All of seven mice in which treatment was begun on the eighth day, succumbed to the trypanosomes. The 76 surviving mice were observed for up to eight months after treatment during which time they appeared to be in perfect health. No parasites were detected in the peripheral blood of these animals, examined with an oil-immersion objective in several hundred stained thick smears. Histological study of the tissues of four of these mice revealed no latent tissue-infection. Furthermore, all the cured mice were immune to

repeated challenges with normally lethal inocula of either WBH-strain or B-strain *T. cruzi*, while an equal number of similarly inoculated non-immunized controls died of the infection.

All of twenty B-strain infected mice survived indefinitely after daily injection with Bayer 7602 from the fifth to the twelfth day, whereas twenty untreated controls died. In contrast with the WBH-series, the treated B-series continued to show a slight residual infection in ten consecutive sets of blood-smears taken for a period of 77 days. These mice were immune to challenge with WBH-strain.

TABLE 1.—*Comparison of tissue-infection in mice produced by two lethal strains of* Trypanosoma cruzi

Tissue or Organ	12 mice infected with *T. cruzi* B-strain		12 mice infected with *T. cruzi* WBH-strain	
	Total found infected	Degree of infection	Total found infected	Degree of infection
Heart	11	++	3	+
Liver	2	++	11	+++
Kidney	1	+	11	++
Spleen	4	++	10	+++
Lung	2	+	6	++
Small Intestine	6	+	5	++
Skeletal Muscle	9	++	12	++
Gonads	0	–	0	–
Brain	0	–	0	–

– = no parasites found in 100 high power fields.
+ = parasites rare.
++ = parasites present in about ¼ the fields examined.
+++ = parasites abundant.

Although they are more virulent, the WBH-trypanosomes are more susceptible to quinoline, than the B-trypanosomes; but despite this strain-specific behavior, there is sufficient immunological cross-relationship to afford complete mutual protection.

4. Response to Seasonal Temperatures

The blood populations of WBH-strain reached their height on the eighth day of infection at which time the trypanosomes were routinely counted in stained thick smears made from heart blood. For the B-strain this was done on the twelfth to to fourteenth day. Figure 2 gives the results for a period of 27 months arranged in quarters.

The specific behavior of the two strains remained constant over this time. Each point in the B- series is based on an average of seven and one-half counts from as many infected mice (total 60), while each point in the WBH-plot represents an average of twenty-three individual thick smears (total 205). Between the spring of 1947 and June 1949 a very gradual increase of about 150 per cent was observed in the blood populations of the B-trypanosomes. Under the same environmental and host-genetic conditions of propagation the WBH-strain counts rose nearly 800 per cent. This enormous increment was, however, not reflected in a significant reduction of survival time (cf. Figure 1).

Superimposed on the overall increase of WBH-populations is a temperature-conditioned seasonal rhythm with an upward trend in spring and fall and a decrease in summer and winter. The number of blood trypanosomes rose with decreasing temperature and declined with rising temperature. During spring and fall, when the official U.S. Weather Bureau averages were below 70° F and the central steam heat

was shut off, the thermometer in the experimental room read 70° F ± 2°. The mean outside readings during the summer months raised this value to the middle 70's. The mean winter indoor temperatures were in the high 70's day and night, heat being supplied from the hospital's central plant. The range of mean temperatures to which the mice were exposed, thus did not exceed 12° F, which makes the seasonal

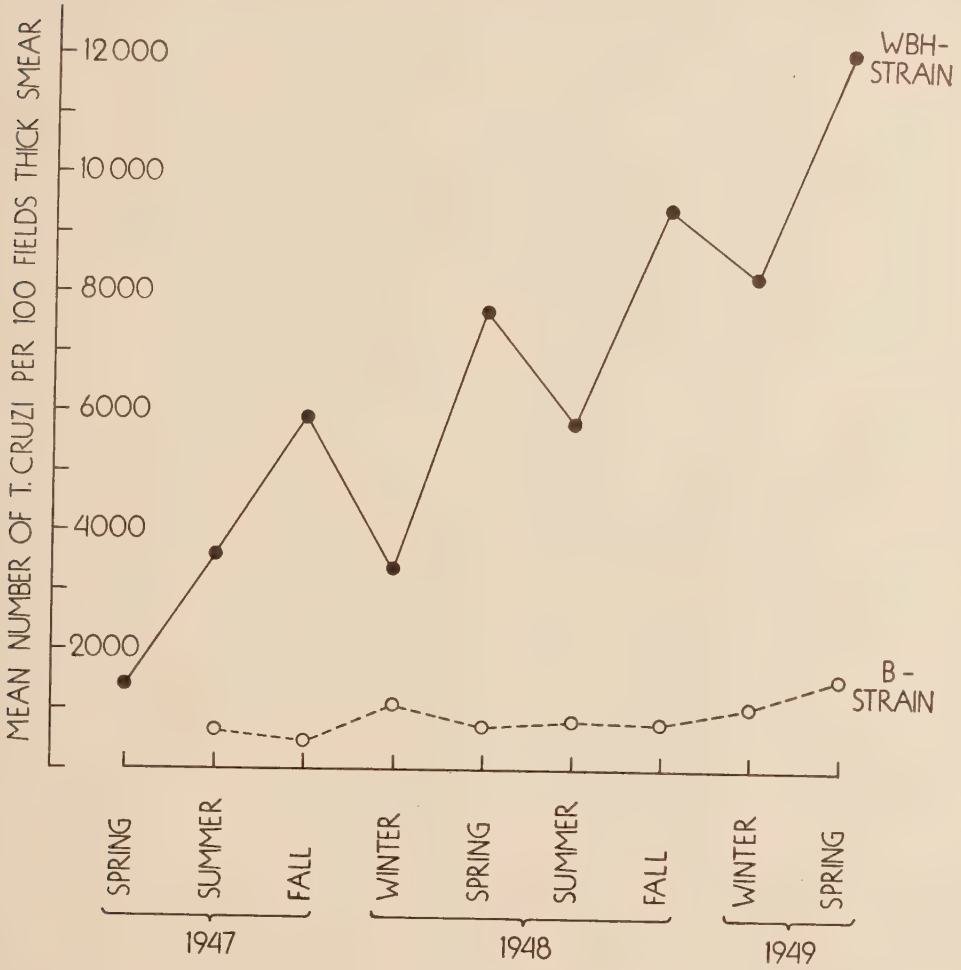

FIG. 2. Influence of seasonal temperatures on blood populations of WBH-strain *T. cruzi*. Absence of seasonal rhythm in B-strain counts.

Based on parasite counts in thick blood smears from 265 infected mice at a magnification of 900 diameters.

fluctuations of the infection all the more remarkable. No seasonal rhythm was noted for the B-strain.

DISCUSSION

Through the use of genetically homogeneous hosts of the same sex and comparable age kept on a standardized diet and infected at regular intervals with uni-

form inocula, the majority of variables which enter into determining virulence levels was eliminated. The results may therefore be interpreted as brought about by the parasite's own strain-specific physiological pattern.

Strain-differences in virulence have, of course, been described for a number of pathogenic Protozoa, for example the several races of *Entamoeba histolytica* (Meleney and Frye, 1935) and the multiple strains of the human malarias (Hackett, 1937; Boyd and Kitchen, 1937). Virulence in *E. histolytica* is, however, complicated by accompanying bacteria. The strains of various species of *Plasmodium* compared for virulence often differed both morphologically and immunologically, while we are here dealing with two strains of *T. cruzi* which are cytologically indistinguishable and immunologically cross-reactive.

The emphasis is, therefore, not on the mere fact of a virulence difference, but on the persistence of this difference after elimination of all controllable environmental variables and on the clearcut upper limits of pathogenicity as measured by host survival time. Each of the two strains, under optimal conditions of prolonged serial passage, showed and maintained its own specific acme of virulence.

The principal reason for this difference lies probably in the preferential tissue affinity of the WBH-trypanosomes for liver and spleen, and the B-trypanosomes for heart muscle. Not only might there be less sugar available for the parasites in active muscle, with consequent reduction in division rate, but the extensive damage done to the spleen by the WBH-strain would result in a general impairment of immune reactions similar in effect to experimental splenectomy. This could account for the enormous blood populations attained by the WBH-strain, against which the antibody obstacle should theoretically be less than against the more slowly growing B-strain.

The curative value of Bayer 7602 continues to be a controversial subject. Mazza (1941, and numerous later papers) has published many case reports showing beneficial results of this drug; but he also admits some failures. Critically controlled tests with 200 mice, on the other hand, have led Fulton (1943) to the conclusion that no complete cures resulted from the use of Bayer 7602 which, besides being rather toxic, had only limited prophylactic possibilities. Our results with B-strain *T. cruzi* confirm Fulton; but the high percentage of cures of the otherwise invariably fatal WBH-infection coupled with the negative findings in numerous thick blood smears and tissues of the treated mice suggest that strain-differences in susceptibility may account for the discrepancies in the published claims pro and con Bayer 7602. It is therefore premature to discontinue clinical trial of Bayer 7602, as seems to be the present tendency in several South American endemic areas.

The rhythmic seasonal fluctuations in numbers of WBH-trypanosomes are in agreement with the report of Kolodny (1939) that young albino rats suffer less intense infections during the summer months than during other seasons. Kolodny (1940) put this observation to an experimental test and concluded that raised environmental temperatures (90°–95° F) increased only slightly the resistance of rats to infection with *T. cruzi,* while a lowered environmental temperature (40°–45° F) generally resulted in heavy bloodstream invasion, several times more intense than usually seen in animals of the same age maintained at room temperature. Under these rather drastic conditions an ordinarily mild infection became exceedingly acute and almost invariably fatal.

Although the temperature changes in the present investigation were never sudden and the range was only about one-third of that tested by Kolodny, the fluctuations for the WBH-strain during 27 consecutive months were large and regular enough to be considered significant. The B-strain blood populations, on the other hand, were not correlated with season. Since the parasite counts for this strain were always much smaller than for the WBH-strain, it appears that the seasonal changes in room temperature (68°–91° F) modify the degree of infection only above a certain strain-inherent threshold of virulence.

SUMMARY

Two lethal, immunologically cross-reactive, and morphologically indistinguishable strains of *Trypanosoma cruzi,* originally isolated from human patients, were maintained for 28 and 41 months respectively by serial transfer through inbred male mice. The two strains exhibited well defined and constant strain-specific levels of virulence, different degrees of affinity for certain host tissues, unequal susceptibility to the quinoline-derivative Bayer 7602, and a difference in response to environmental temperature.

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A NEW TAPEWORM OF THE GENUS *BOTHRIOCEPHALUS* FROM OKLAHOMA SALAMANDERS*

J. D. Reeves

The worms used in the present study comprise eight strobilae having scoleces, and a number of fragments. Four of the parasites were found in each of two out of three larval specimens of *Typhlotriton spelaeus* Stejneger, 1892,[1] taken from a spring just below the dam of Grand Lake near Disney, Mayes County, Oklahoma. The salamanders were collected by Professor George A. Moore's class in ichthyology in April 1948. The cestodes were fixed in hot Bouin's fluid, stained in borax carmine, and mounted *in toto* in Canada balsam.

Bothriocephalus typhlotritonis n. sp.
(Figs. 1–14)

Description and measurements entirely from preserved material. Largest complete strobila about 40×0.83 mm, with 118 proglottids. Scolex slenderly elliptical, somewhat narrowed posteriorly, almost neckless, with a faint suggestion of a broad flat apical prominence which becomes thicker, more domelike, and much more pronounced when the shallow lateral bothria are everted. Five scoleces measure 520 (371–663) ×233 (186–265) μ. In determining length the first distinct line of segmentation was used as the posterior limit of the scolex—an unsatisfactory landmark for obviously in life the length would vary back and forth as new segmentation lines form and recede posteriad. Apparent width likewise varies according to whether the bothria are retracted or everted. In two specimens with retracted bothria the grooves measure averagely and respectively 265 and 398 μ in length and 41 and 21 μ in depth, the second scolex being the more stretched longitudinally.

Strobila thin, flat, and moderately notched marginally between segments. Proglottids show a pronounced though not entirely consistent tendency to occur in couplets, the anterior segment in the couplet being generally distinctly the smaller and the interproglottidal boundaries and marginal notches being usually more pronounced between couplets than within them. This grouping of segments, commonplace among species of *Bothriocephalus,* has been attributed (Van Cleave and Mueller, 1934) to "secondary fragmentation" of primary proglottids. In five strobilae the most-anteriad distinct segment measures 255 (66–325) ×157 (133–172) μ. Largest segment in all our material (third from rear in Fig. 12) 852×828 μ—slightly longer than wide. *Anlagen* of central reproductive organs (cirrus pouch, ovary, uterus, egg sac) begin to appear at about 45 segments back of scolex. Testes do not appear until later but are first to become fully formed—at about 85 segments; the central organs become fully differentiated at about the 100th segment.

Testes 55–85 in number, spheroidal to broadly ellipsoidal, rather smooth in outline, from 15 μ in short diameter to 25 μ in long diameter; distributed in distinctly separated lateral fields occupying almost entire length of segment; each lateral field vaguely subdivided by locus of longitudinal nerve into marginal and medial groups—the marginal testes outnumbering the medial about 3 to 1; mature and clearly discernible in advance of the other reproductive structures, thereafter gradually becoming vacuous, faint-staining, and increasingly indistinct—being further obscured by the much more numerous and much more heavy-staining vitellaria. Vasa efferentia not observed. Portion of vas deferens leading to cirrus pouch swollen, sperm-filled, and convoluted into a conspicuous mass located to right or left (alternating irregularly from segment to segment) of median line and ventrolateral to pouch. Cirrus pouch spheroidal, submedian, and slightly postequatorial; 66–93 μ in diameter; receives spermatoza ventrally and discharges dorsally via a short slender sinuous canal into the small vertically placed common genital atrium; 66–93 μ in diameter. Cirrus scarcely discernible in our whole mounts; not observed in extruded condition.

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* Contribution No. 149 from the Department of Zoology, Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma; prepared under the direction of R. Chester Hughes. Professor Bryan P. Glass, of the same department, identified the salamanders.

[1] Since this paper went to press, the two host individuals were restudied after being stained with alizarin; one proved to be *T. spelaeus* and the other *T. nereus* Bishop, 1944.

Ovary near posterior side of segment; generally kidney-bean-shaped with concave side forward and usually slightly bilobate with principal lobes further extensively subdivided; from 233 to 237 μ in transverse diameter and 99 to 113 μ long in median line of proglottid. Vitelline reservoir of variable shape and size situated shortly in front of ovary. Mehlis' gland ventral to yolk reservoir. Vagina thick-walled and slightly sinuous; leads almost directly posteriad from genital atrium. Uterus thick-walled with large extensively convoluted lumen; follows a roughly S-shaped path forward from side of Mehlis' gland ipsilateral with vas deferens, thence past opposite side of cirrus pouch; expands abruptly near its distal end to form a large submedian and usually slightly preequatorial, spheroidal or ellipsoidal, egg sac (73–119 μ in short diameter by 133–199 μ in long diameter—direction of long axis having seemingly no constant relationship to that of strobila) which discharges ventrally through a short canal and the uterine pore. Vitellaria exceedingly numerous and very widely distributed, occupying almost the whole of the cortical parenchyma; the distribution being somewhat interrupted in the median areas adjacent to the central reproductive organs; because of their extraordinary numerosity, extensive overlapping, and irregular form, they defy accurate counting but the number approaches 1000 in larger segments[2]; roughly spheroidal to ellipsoidal and very irregularly lobulated; 15–25 μ in diameter.

Eggs operculate, narrowly ellipsoidal, 51.5 (43.5–52.5) × 32.5 (31.5–33) μ; begin to appear with fully formed shells at about segment 110 back of scolex; generally fewer than 10 per uterus although as many as 75 occur.

Host. *Typhlotriton spelaeus* Stejneger, 1892, and *T. nereus* Bishop, 1944.

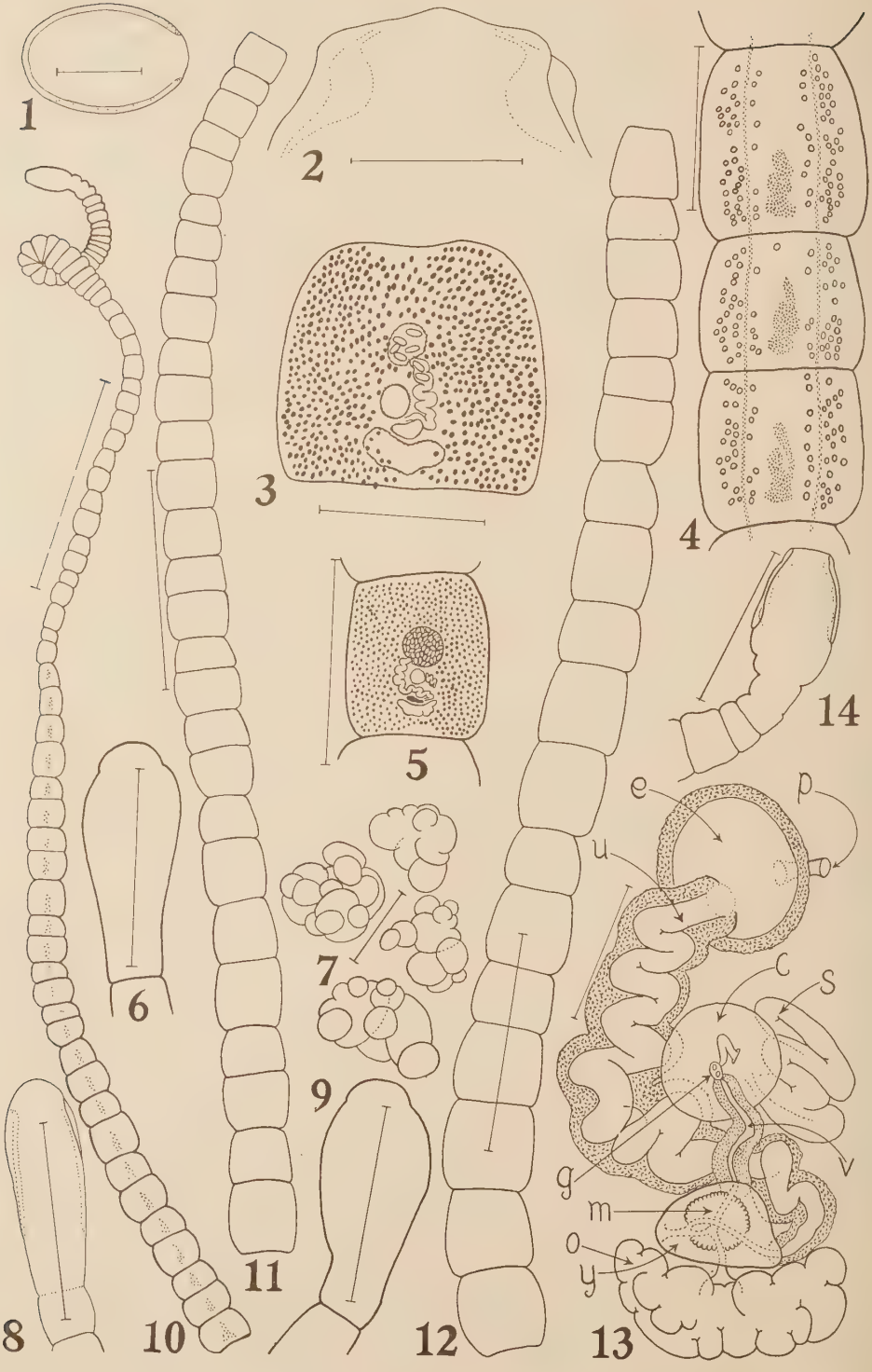
Locality. Grand Lake near Disney, Mayes County, Oklahoma.

Cotype specimens. Seven microscope slides bearing *in toto* preparations of 5 scoleces and proglottids of various ages; U. S. Nat. Mus. Helm. Coll. No. 37129.

Joyeux and Baer (1936) place the genus *Bothriocephalus* Rudolphi, 1808, in the family Ptychobothriidae Lühe, 1902, and the order Pseudophyllidea Carus, 1863. Like so many other old generic names, *Bothriocephalus* has been used as a catchall for a congeries of diverse forms. A large percentage of these were larvae found in piscine hosts and for which the respective adult worms were either unknown or indeterminable at the time. Subsequently, as taxonomic relationships and life histories have become better understood, many of these have been shifted to other genera. It was pointed out by Thomas (1937a) and others that probably no species developing definitively in birds or mammals belongs properly in *Bothriocephalus;* and by Hughes, Baker, and Dawson (1941) that the several forms (either larval or adult) reported as *Bothriocephalus* from reptiles also belong elsewhere. Many definitively piscine species have also been removed to other genera. In modern characterizations *Bothriocephalus* embraces tapeworms having adults (Joyeux and Baer, 1936) "chez les Téléostéens" or (Thomas, 1937a) "in fish and amphibia." In discussing species of the genus, Joyeux and Baer state: "Les caractères indiqués par les divers auteurs sont si vagues et si peu constants qu'il nous est impossible de faire une clé pour différencier les espèces les unes des autres. Nous attirons l'attention des Zoologistes sur l'état chaotique du genre . . ." In view of this state of affairs it seems pointless for us to present detailed comparisons between our species and the forms parasitic in marine and exotic hosts, all of which occur definitively in fish.

According to Van Cleave and Mueller (1934) the species of *Bothriocephalus* found in American fresh-water fishes include *B. claviceps* (Göze, 1782) Rudolphi,

[2] In response to query, Professor Lyell J. Thomas, University of Illinois, has very kindly informed us by letter that his statement (Thomas 1937a: 123) that the vitellaria in *B. rarus* number 210–750 was intended to read 210–250. Twice elsewhere in the same article (pp. 120, 126) the number is expressed as 210–250.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Explanation of Plate

The figures all concern *Bothriocephalus typhlotritonis* n. sp. They were all made with the aid either of a camera lucida or a projection apparatus, minor details and corrections being supplied freehandedly. Abbreviations: *c,* cirrus pouch; *e,* egg sac; *g,* common genital pore and atrium; *m,* Mehlis' gland; *o,* ovary; *p,* uterine pore; *s,* vas deferens; *u,* uterus; *v,* vagina; *y,* vitelline reservoir.

Fig. 1. Egg. Scale, 25 μ.

Fig. 2. Oil-immersion study of front end of scolex shown in Fig. 14. Medial lines represent depths of bothria. Scale, 100 μ.

Fig. 3. Proglottid to show vitellaria. The follicles are not all at the same focal depth and considerable overlapping occurs. Near the margins the glands are all shown, but in the central region only those in the upper cortex are represented. Over 600 follicles are depicted but the segment actually contains a great many more. Note 7 eggs *in utero.* Scale, 500 μ.

Fig. 4. Three consecutive segments to show variation in number and arrangement of testes. Here the testes have reached maximum clarity but the central reproductive organs are not yet fully differentiated. Scale, 500 μ.

Fig. 5. Segment with fully filled egg sac; eggs not all shown, there being considerable overlapping. Scale, 1 mm.

Figs. 6, 9. Scoleces with bothria everted causing apical protuberances to appear more pronounced. Scale, 500 μ.

Fig. 7. Four vitelline follicles. Scale, 25 μ.

Figs. 8, 14. Scoleces in different states of contraction and with noneverted bothria. Scale, 500 μ.

Figs. 10, 11, 12. Our largest and longest complete strobila, shown in three sections. Scale, 2 mm.

Fig. 13. Central reproductive structures, dorsal view. Scale, 100 μ.

1810; *B. cuspidatus* Cooper, 1917; and *B. formosus* Mueller and Van Cleave, 1932. The only previously known amphibian species is *B. rarus* which was described from *Triturus viridescens* in Michigan by Thomas (1937a). Thomas (1937b) also studied the life cycle of this form. The above-mentioned species differ from *Bothriocephalus typhlotritonis* n. sp. chiefly as follows.

B. claviceps, as described by Cooper (1919), is very much larger generally and has relatively larger bothria; a very prominent apical scolecid disc; posterior segments much wider than long; averagely fewer testes; fewer vitellaria; egg sacs relatively larger, more irregular in shape, and more transversely elongate; and eggs much larger, nonoperculate, and much more numerous *in utero.*

B. cuspidatus, as described by Cooper (1917, 1919), is also larger (though smaller than *B. claviceps*) and has the bothria deepest posteriorly; a very prominent, surficially notched, apical, scolecid disc; posteriormost segments 2 to 4.5 times as wide as long; testes averagely fewer and much larger; eggs and vitellaria much larger; and egg sacs and uterine pores farther anteriad.

B. formosus, as described and figured by Mueller and Van Cleave (1932) and Van Cleave and Mueller (1934), has a shorter and wider strobila; a smaller scolex; older proglottids generally wider than long; egg sacs and uterine pores farther forward; eggs, vitellaria, and testes relatively larger; and eggs *in utero* more numerous.

B. rarus, the only other species known from batrachians, has the strobila and scolex both considerably larger, averagely fewer testes, much fewer vitellaria, smaller ovaries, actually and relatively much larger gravid egg sacs, and eggs considerably larger and more numerous per uterus.

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NOTES ON THE MORPHOLOGY AND LIFE CYCLE OF THE GENUS *MONOECOCESTUS* BEDDARD, 1914 (CESTODA: ANOPLOCEPHALIDAE) FROM THE PORCUPINE[1,2]

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Over the past three years cestodes from the intestines of 17 Canada porcupines, *Erethizon dorsatum dorsatum* (Linnaeus, 1758), collected from various parts of northern Minnesota have been examined. Morphological comparison of these cestodes with existing descriptions revealed several details in these descriptions which should be modified. In addition, some observations on the biology and life cycle of these worms have been made.

Of the cestodes recorded from North American porcupines, only two species have had repeated verification. Most workers have placed these worms in the genus *Schizotaenia* Janicki, 1904 (see Douthitt, 1915; Baer, 1927; Jellison, 1933; Chandler, 1936; and Rankin, 1946). Apparently the later American workers had overlooked the paper by Fuhrmann (1932) in which he pointed out that *Schizotaenia* Janicki, 1904 (Cestoda) was preoccupied by *Schizotaenia* Cook, 1895 (Myriapoda). Fuhrmann reinstated *Monoecocestus* Beddard, 1914 as the proper name of this genus. The specific identification of these two cestodes has occurred with several names and combinations; a complete list of synonyms is included later with a redescription of each species.

Two other genera have been reported from the intestines of North American porcupines. Leidy (1855) gave a short description of a cestode, *Taenia laticephala,* from *E. dorsatum,* but he did not give sufficient detail for recognition of a present-day genus, and since no type material has been found, Baer (1925) considered *Taenia laticephala* Leidy, 1855 a *nomen nudum.* Cobbold (1862) reported a cestode from *E. dorsatum* which he identified as *Taenia pectinata* Goeze, 1782; this record was later included by Meggitt (1924) under *Cittotaenia pectinata* (Goeze, 1782). As early as 1891, Blanchard concluded that this record was based on a doubtful diagnosis. Since no further records of *Cittotaenia* from porcupines have occurred in the literature, it appears that Blanchard's doubts were correct.

Two larval cestodes have been reported from North American porcupines. Schwartz (1924) described a proliferating larval tapeworm, *Taenia twitchelli,* from the lung of an *E. epixanthum* from Alaska; McIntosh (1938) showed that the adult of this larva occurs in the Alaskan wolverine. Gower (1939) described another larval tapeworm, *Taenia michiganensis,* from the liver of an *E. dorsatum* from Michigan.

For a complete history of the naming as well as for the existing figures and descriptions of *Monoecocestus* in porcupines, one must refer to Leidy (1855), Stiles (1895, 1896), Stiles and Hassall (1902), Janicki (1904, 1906), Cohn (1906),

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[1] Paper No. 2474 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

[2] The author wishes to extend his thanks to Dr. Franklin G. Wallace under whose guidance this study was made, and to Dr. E. W. Price of the Bureau of Animal Industry for the use of type material and for certain suggestions, as well as to others who aided in this study.

Beddard (1914), Douthitt (1915), Meggitt (1924), Baer (1925, 1927), Fuhrmann (1932) and Chandler (1936).

Although it is not the purpose of this paper to cover the distribution of the species and subspecies of porcupines in North America, it is of interest to note that Anderson and Rand (1943) concluded that only one species of porcupine (*Erethizon dorsatum*) with five forms is distributed through Canada and Alaska. On the basis of their work, all parasite records from *E. epixanthum* from Alaska should then be listed as from *E. dorsatum myops*. They have *E. epixanthum* with two forms restricted to Nebraska, Wyoming, Arizona (and Colorado?), while *E. dorsatum epixanthum* is confined to the general area west of the Rocky Mountains in the United States. In the United States, *E. dorsatum dorsatum* is confined to the region extending from the northeastern portion of the country through the states bordering along Canada as far west as Minnesota, while this subspecies has a wide range through southern and midwestern Canada. Although Anderson and Rand are a little vague regarding other subspecies found in Minnesota, Swanson, Surber and Roberts (1945) state that only *E. d. dorsatum* has been reported from Minnesota. On the basis of the work just cited, the majority of the cestode records to be found in the literature would come from *E. dorsatum*. However, apparently the cestodes reported by Chandler (1936) did come from one of the forms of *E. epixanthum* since that animal was taken in Colorado, while some specimens examined by Douthitt (1915) came from an *E. epixanthum* from Wyoming.

MORPHOLOGY AND DIAGNOSES

Although Chandler found that the two species of *Monoecocestus* from *E. epixanthum* are readily differentiated with the naked eye, we found we could not do this with the specimens from *E. dorsatum*. Chandler characterized *M. americanus* as being 30 to 60 mm. long with a maximum width of 3 to 4.5 mm. and with the ovaries broadly overlapping, while the other, *M. variabilis*, was 33 to 42 mm. long with a maximum width of 7.5 to 9.5 mm. and with the ovaries rarely touching the midline of the worm. Of particular interest is the fact that we have taken worms up to 270 mm. in length and 12 mm. in width from *E. dorsatum*, while on the average, even in the heaviest infections, the worms have averaged 2 to 3 times as large as previously reported. The length/width relationships of either the whole worm or of individual mature proglottids has not been found a reliable criterion for separating the two species.

In most of these worms the position of the ovary in respect to the midline of the worm gave a reliable species separation, but some doubtful specimens were found where a clear-cut species differentiation could not be made on this basis. The width/length ratio of the ovary was computed, and although the mean of the ratios for each species gave a definite separation, there was sufficient overlapping of extreme cases so that this relationship alone could not be used. It was found that separation of species could be based on the relationship of (1) the width/length ratio of the ovary to (2) the distance of aporal displacement of the midline of the ovary from the

[3] The region of sexual maturity as interpreted here was composed of those proglottids, usually around 5 in number, containing the majority of the fully developed reproductive organs and lying immediately anterior to the point where the uterus became visible in whole mount. Generally this region was between the 50th to the 70th proglottids in both species, although it was found to occur as early as the 35th proglottid, or as late as the 126th proglottid.

midline of the proglottid at sexual maturity.[3] With all measurements to the nearest 0.01 mm., the width of the ovary divided by its length gave a quotient. This quotient divided by the distance between the midline of the ovary and the midline of the proglottid gave a figure, here called the ovarian index. In all, 91 separate whole mounts of these worms were measured. In *M. americanus* the ovarian index ranged from 4.1 to 23.5, with a mean of 11.5, while in *M. variabilis* the range was from 1.2 to 3.2, with a mean of 2.3. The figure 3.9 was taken as the dividing point: an index more than 3.9 indicates *M. americanus,* one less than 3.9 indicates *M. variabilis.*

Much the same relationship was found to exist between the width of the ovary and the width of the proglottid. Again with all measurements to the nearest 0.01 mm., if the width of the ovary were divided by the width of the proglottid, and the quotient thus obtained divided by the distance between the midline of the ovary and the midline of the proglottid, a quotient, here called the ovary-proglottid index, was obtained. In *M. americanus* this index ranged from 0.4 to 3.3, with a mean of 1.3, while in *M. variabilis* the range was from 4.0 to 9.4, with a mean of 6.1. Again 3.9 was taken as the dividing point: an index of more than 3.9 indicates *M. variabilis,* one less than 3.9 indicates *M. americanus.* Notice that these figures are just the reverse of the figures for the ovarian index.

New diagnoses, based on present material as well as a study of type specimens are given below.

Family ANOPLOCEPHALIDAE Fuhrmann, 1907

Subfamily ANOPLOCEPHALINAE Blanchard, 1891

Genus *Monoecocestus* Beddard, 1914 (modified from Baer, 1927); Anoplocephalinae. Mature strobila average in shape. Genital pores regularly or irregularly alternate. Vagina and cirrus sac dorsal to excretory canals and lateral nerve. Excretory system with or without numerous anastomoses. Testes numerous, located posteriorly in proglottid in a transverse field. Cirrus well developed, armed with spines. Vagina anterior to cirrus sac. Ovary median or poral. Uterus diffusely reticulate or sacciform. Eggs with pyriform apparatus. Adults in rodents or artiodactyles. Type species: *Monoecocestus hagmanni* (Janicki, 1904) n. comb.[4]

MONOECOCESTUS AMERICANUS (Stiles, 1895) Fuhrmann, 1932.[5]

Synonyms:

Taenia laticephala Leidy, 1855 (according to Cohn, 1906)
Andrya americana Stiles, 1895
Bertia americana (Stiles, 1895) Stiles, 1896
Bertiella americana (Stiles, 1895) Stiles and Hassall, 1902
Bertia laticephala (Leidy, 1855) Cohn, 1906
Schizotaenia americana (Stiles, 1895) Janicki, 1906
Monoecocestus erethizontis Beddard, 1914
Schizotaenia laticephala (Leidy, 1855) Meggitt, 1924
Monoecocestus erethizonii Beddard, 1914 (from Meggitt, 1924)

Hosts:

Erethizon dorsatum (Linnaeus, 1758), and probably all subspecies.
Erethizon epixanthum Brandt, 1835, and probably all subspecies.

[4] Hughes (1941) pointed out that *Taenia decrescens* Diesing 1856, the worm which Janicki (1904) designated as type for his new genus *Schizotaenia,* was a homonym of *Taenia decrescens* Rudolphi (from Creplin, 1849) and proposed the new name *Schizotaenia diesingi* Hughes, 1941. Apparently, Hughes overlooked the fact that Baer (1927) had reduced the name *Schizotaenia hagmanni* Janicki, 1904 to a synonym of *S. decrescens.* Therefore, the creation of the specific name *diesingi* was not justified.

[5] Fuhrmann (1932) has a mislabeled figure in his text (p. 67, Fig. 35). The figure (taken from Douthitt, 1915) and labeled *Monoecocestus americanus* should be *Monoecocestus variabilis.*

Distribution:
North America
Intermediate hosts:
Mites of superfamily Oribatoidea
Diagnosis. *Monoecocestus.* Adult 33 to 270 mm. in length by 5.0 to 12.0 mm. in width; strobila with 90 to 200 proglottids. Scolex 0.5 to 0.6 mm. in diameter; suckers 0.16 to 0.24 mm. in diameter. Genital pores alternate, generally regularly, occasionally irregularly. Excretory system occasionally with, but generally without, anastomoses. Testes numbering from 50 to 103, dorsal, confined to a bilaterally symmetrical field with the lateral margins approximately equidistant from the ventral excretory canals. Testes spherical, elongate or irregular, from 0.04 to 0.10 mm. in maximum diameter. Cirrus sac pyriform, from 0.43 to 0.95 mm. in length by 0.14 to 0.24 mm. in diameter. Functional vagina generally in 5th to 10th proglottid posterior to scolex, opening into genital sinus, at sexual maturity anterior and slightly ventral to cirrus sac, not contiguous with seminal receptacle. Ovary lobed, 0.36 to 0.71 mm. in length by 0.81 to 2.13 mm. in width, ventral, displaced from midline of worm but generally with aporal margins overlapping in succeeding proglottids. Approximately two thirds of ovary poral to longitudinal line through center of shell gland. Ovarian index more than 3.9; ovary-proglottid index less than 3.9 (see above). Vitelline and shell gland complex generally with posterior margin posterior to postero-lateral margins of ovary. Uterus confined medial to the ventral excretory canals, a reticulum; in the most posterior proglottids may be sacculate or a simple sac. Hexacanth embryo from 0.012 to 0.016 mm. in diameter, enclosed in 3 coats, the innermost coat being the pyriform apparatus, outer coat covered with minute spines.

MONOECOCESTUS VARIABILIS (Douthitt, 1915) n. comb.

Synonyms:
Monoecocestus erethizontis Beddard, 1914 (according to Baer, 1927)
Schizotaenia variabilis Douthitt, 1915
Schizotaenia erethizontis (Beddard, 1914) Baer, 1925
Hosts:
Erethizon dorsatum (Linnaeus, 1758), and probably all subspecies.
Erethizon epixanthum Brandt, 1835, and probably all subspecies.
Distribution:
North America
Intermediate hosts:
Mites of superfamily Oribatoidea
Diagnosis. *Monoecocestus.* Adult 20 to 174 mm. in length by 5.0 to 12.0 mm. in width; strobila with 60 to 164 proglottids. Scolex 0.5 to 0.6 mm. in diameter; suckers 0.16 to 0.26 mm. in diameter. Genital pores alternate, generally regularly, occasionally irregularly. Excretory system occasionally with, but generally without, anastomoses. Testes numbering from 56 to 128, dorsal, confined to an elongate teardrop-shaped field with the distance from the narrowed aporal end to the aporal ventral excretory canal approximately twice as great as the distance from the broadened poral end to the poral ventral excretory canal. Testes spherical, elongate or irregular, from 0.04 to 0.08 mm. in maximum diameter. Cirrus sac pyriform, from 0.50 to 0.85 mm. in length by 0.14 to 0.25 mm. in diameter. Functional vagina generally in 15th to 20th proglottid posterior to scolex, opening into genital sinus, at sexual maturity anterior and slightly ventral to cirrus sac, not contiguous with seminal receptacle Ovary lobed, 0.36 to 0.74 mm. in length by 0.57 to 1.85 mm. in width, ventral, with aporal margin rarely reaching midline of worm. Ovary divided into approximately equal parts by a longitudinal line through center of shell gland. Ovarian index less than 3.9; ovary-proglottid index 3.9 or greater (see above). Vitelline and shell gland complex generally with posterior margin slightly anterior to postero-lateral margins of ovary. Uterus confined medial to the ventral excretory canals, a reticulum; in the most posterior proglottids may be a sparse reticulum or sacculate. Embryonated egg indistinguishable from *M. americanus.*

For a quick diagnosis, the following short key based on proglottids at sexual maturity will give a satisfactory separation of species.

1). Ovaries broadly overlapping in succeeding proglottids ..... *M. americanus.*
2). Ovaries definitely displaced from midline of worm ..... *M. variabilis.*
3). Ovaries just overlapping in succeeding proglottids, or slightly displaced from midline of worm.

a) Ovarian index greater than 3.9; ovary-proglottid index less than 3.9; testis field bilaterally symmetrical with ends of field equidistant from ventral excretory canals ..... *M. americanus.*

b) Ovarian index less than 3.9; ovary-proglottid index greater than 3.9; testis field of an elongate tear-drop shape and the distance from the narrowed aporal end to aporal ventral excretory canal twice as great as distance from broadened poral end to the poral excretory canal ..... *M. variabilis.*

In both species the vagina showed a development not usual among the cyclophyllidean cestodes. Although Beddard (1914) gave a good description of certain aspects of the vaginal development, he apparently failed to realize the significance of this development; other workers have had inadequate descriptions or have completely omitted the vaginal development in their description, so a more adequate description is included here.

In *M. americanus* the vagina was a complete tube by the 5th to the 10th proglottid posterior to the scolex, while this generally occurred by the 15th to the 20th proglottid in *M. variabilis.* In these proglottids the vagina was greatly enlarged. From the position anterior and slightly ventral to the primordial cirrus where the vagina opened into the genital sinus, it extended mediad, passing ventral to the cirrus but dorsal to the excretory canals, until it joined the large empty seminal receptacle. One or two proglottids posterior to the one with the enlarged complete vagina, the seminal receptacle was found to contain sperm, and a few more proglottids posteriorly the vagina was already in a process of degeneration with the distal and proximal portions of the vagina no longer contiguous (see Fig. 1). Since at this point in the strobila all other reproductive organs were, at most, only differentiable as primordia, it seemed safe to assume that cross-fertilization by a more mature proglottid, in either the same or another strobila, had taken place. Since no worms, or proglottids, were observed in copula, this point could not be verified.

The above described condition of the vagina was in contrast with the condition found at maturity where the vagina had greatly atrophied. In *M. americanus* only a short remnant of a tubule extending porad from the seminal receptacle was visible, while in *M. variabilis* in addition to this short tubule another fragment anterior to the cirrus could generally be seen.

The embryonated egg of these species has not been adequately described previously. The eggs of the two species were identical, and although of the anoplocephalid type in that they possessed 3 coats, or shells, of which the innermost was pyriform in shape, they appeared to be different from the other described species in this family by having the outer coat covered with minute spines about 0.003 mm. long (see Fig. 2). These spines were always present on eggs taken from an unpreserved gravid proglottid, but were occasionally absent from eggs found in the host feces. The spines were not observed on eggs from gravid proglottids after preservation in 5% formaldehyde or 70% alcohol, which might account for their not being reported by earlier workers. Baer (1927) reported the eggs of *M. americanus* as varying from 0.055 to 0.061 mm. in diameter, and eggs of *M. variabilis* as poorly known; in the present study the unpreserved eggs of both species were slightly larger than Baer reported, and eggs up to 0.075 mm. in diameter were not uncommon. The middle coat, usually subspherical or occasionally irregular in

shape, was approximately 0.030 mm. in diameter, and usually in contact with the outer coat along a portion of its margin. Usually the cavity between the outer and middle coats was filled with many calcareous granules which made observation of internal details difficult, but occasionally an egg that showed the structure of the pyriform body and its contained embryo could be found. The inner coat was pyriform in shape and around 0.020 mm. in its longest dimension and 0.017 mm. across the globose portion. The narrower end of this pyriform apparatus ended in two blunt horns with what looked like a single long filament extending from each horn. The filaments were free in the cavity between the inner and middle coats, and appeared to wrap themselves around the pyriform apparatus in a loose and irregular fashion. The hexacanth embryo varied from 0.012 to 0.016 mm. in diameter and had 6 hooks. The hooks measured close to 0.010 mm. in length although very rarely hooks as short as 0.008 mm. or as long as 0.012 mm. were found (see Fig. 3).

LIFE CYCLE STUDIES

By the way of a preliminary note, a few observations regarding the life cycle of these two species of cestodes will be included here. A detailed report will be published at a later date.

Since Stunkard (1937) first reported that *Moniezia expansa* was capable of utilizing oribatid mites as intermediate hosts, several other anoplocephalid cestodes have been shown to develop in these interesting little animals. Kates and Runkel (1948) gave in tabular form a review of the work that had been published up to that time regarding the role of oribatid mites in anoplocephalid life cycles. To the nine species from the six genera *Moniezia, Bertiella, Cittotaenia, Anoplocephala, Paranoplocephala* and *Thysaniezia* which they list, can now be added *Monoecocestus americanus* and *M. variabilis*. Rendtorff (1948) found that eggs of *Oöchoristica ratti*, although they did not develop in the oribatid mite *Galumna* sp., did develop into infective cysticercoids in members of the insect orders COLEOPTERA and LEPIDOPTERA.

Specimens of the oribatid mites in which cysticercoids of *M. americanus* and *M. variabilis* had been found, have been sent to Dr. Edward W. Baker of the Division of Insect Identification, Bureau of Entomology and Plant Quarantine who has kindly consented to identify them. In a preliminary note, Dr. Baker has informed the author that at least eight families with 12 species from 11 genera appear to be involved. Since Dr. Baker feels that certain genera should be revised and that a number of the specimens are apparently undescribed, further identification of these mites cannot be included here.

Of the 12 species of mites in which cysticercoids have been found, five species were found naturally infected with cysticercoids identified as *Monoecocestus* on the morphology of the embryonic hooks, while 10 species were laboratory-infected. Attempts to infect two species of mites that were found naturally infected have not been successful to date. Studies are being continued on the morphology of the developing cysticercoids, and the length of time required for full development. Attempts to produce an infection with cysticercoids from laboratory-infected mites have not been successful to date, mainly because as yet no satisfactory uninfected definitive host has been available for experimentation.

EXPLANATION OF FIGURES

FIG. 1. *Monoecocestus americanus* Proglottids 5, 6 and 7 posterior to the scolex, showing development of vagina.

FIG. 2. *Monoecocestus americanus* Embryonated egg.

FIG. 3. *Monoecocestus americanus* Hook from embryo in Fig. 2. C.P.′—primordial cirrus pouch; G. Pap.—genital papilla; G.P.—genital pore; G.S.—genital sinus; S.G.′—primordial shell gland; S.R.—seminal receptacle with sperm; S.R.′—primordial seminal receptacle; T.′—primordial testes; V.—functional vagina; V.′—primordial vagina; V.″—degenerating vagina; V.G.′—primordial vitelline gland.

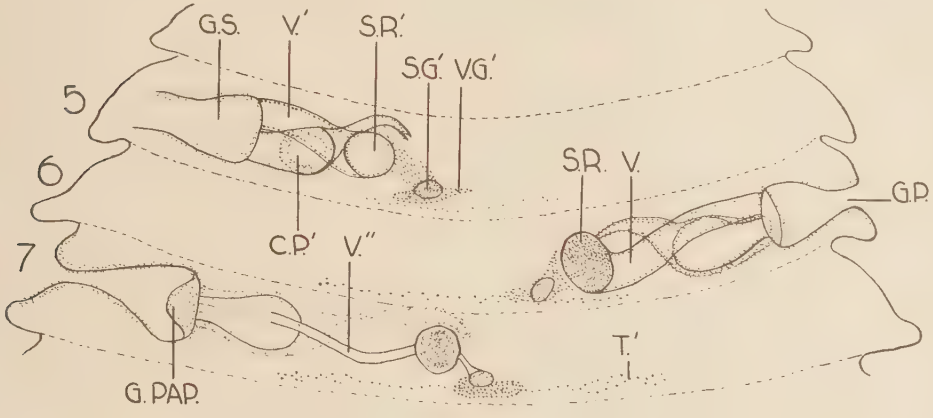

FIG. 1

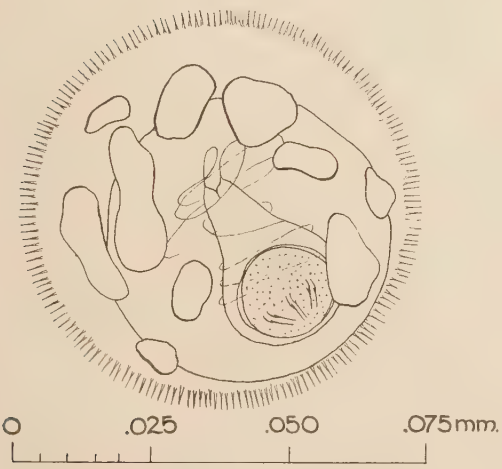

FIG. 2

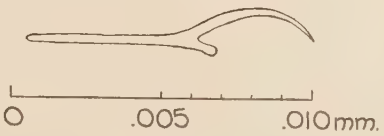

FIG. 3

SUMMARY

1. A brief history of the cestodes reported from North American porcupines is given.

2. A redescription of the genus *Monoecocestus* Beddard, 1914 and the two species of cestodes *Monoecocestus americanus* (Stiles, 1895) Fuhrmann, 1932 and

Monoccocestus variabilis (Douthitt, 1915) commonly found in *Erethizon dorsatum* and *E. epixanthum* is presented.

3. It is shown that three new morphological characteristics, a) the ovarian index, b) the ovary-proglottid index and c) the position and shape of the testis field were a major aid in the separation of doubtful specimens of these two worms.

4. Additional observations on the vagina and embryonated egg of these two species are recorded.

5. In conclusion a preliminary note on the development of these two species of cestodes in at least 12 species of oribatid mites representing eight families is included.

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DIPHYLLOBOTHRIUM STEMMACEPHALUM COBBOLD, 1858 AND *D. LATUM* (LINN., 1758)

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INTRODUCTION

The systematic position and nomenclature of the broad tapeworm of man have been in a state of uncertainty and confusion for more than a century. The species has been shifted from one genus to another until it has become a veritable football of helminthology, to the annoyance of zoologists and physicians. Named *Taenia lata* by Linnaeus (1758); it was transferred by Zeder (1803) to *Halysis* Zeder, 1803 (type, *Taenia solium*) and therefore a synonym of *Taenia*. Study of the scolex showed that the species could not properly be retained with the taenioid cestodes and Bremser (1819) removed it to the genus *Bothriocephalus* Rudolphi, 1808 (type, *Taenia punctata* Rud., 1802; = *T. scorpii* Mueller, 1776 renamed), a group of tapeworms that occurs chiefly in marine teleosts. Blainville (1824) included *Bothriocephalus latus* in the genus *Rhytis* Zeder, 1803 (type *Taenia nodulosa* Pallas, 1781), a species previously named by Rudolphi (1793) as type of the genus *Triaenophorus*. Blainville misspelled the name *"Rhythis"* but stated: "Je réserve ce nom aux bothriocéphales qui n'ont que deux fossettes peu marquées, comme le b. de l'homme (*T. lata*)."

Lühe (1899) stated that the broad tapeworm of man is not congeneric with *Bothriocephalus punctatus* and erected a new genus, *Dibothriocephalus*, with *D. latus* as type. The following species were transferred from *Bothriocephalus* to the new genus: *D. cordatus* (Leuckart, 1863) from man and dogs in Greenland; *D. cristatus* (Davaine, 1893) from man in France; *D. decipiens* (Diesing, 1850) (= *Bothriocephalus felis* Creplin, 1825 renamed; also *B. maculatus* Leuckart, 1848), from domestic cats in Germany and various felines in Europe and South America; *D. dendriticus* (Nitzsch, 1824) from *Larus* spp. in Europe; *D. ditremus* (Creplin, 1825) from *Mergus serrator* and *Colymbus rufogularis* in Europe; *D. felis* (Creplin, 1825) from domestic cats in Germany; *D. fuscus* (Krabbe, 1865) from the dog in Iceland; *D. hians* (Diesing, 1850) (=*Bothriocephalus phocae-foetidae* Creplin, 1825 and *B. tetrapterus* Siebold, 1848 renamed) from *Phoca* spp. in European waters; *D. maculatus* (Leuckart, 1848) from an unidentified leopard; *D. schistochilos* (Germanos, 1895) from *Phoca barbata* near Spitzbergen; *D. variabilis* (Krabbe, 1865) from *Phoca cristata* in European waters; and provisionally, *Bothriocephalus polycalceolus* Ariola, 1896 from *Phoca vitulina*. The genus as erected contained species parasitic in man, in various terrestrial and marine mammals, and in three orders of piscivorous birds. The family Bothriocephalidae Cobbold, 1864 was recognized with five subfamilies: Triaenophorinae, Ptychobothriinae, Dibothriocephalinae, Ligulinae, and Cyathocephalinae. In the subfamily Dibothriocephalinae Lühe included six genera: *Dibothriocephalus* (type, *latus* Linnaeus, 1758;) *Duthiersia* (type, *fimbriata* Diesing, 1854); *Scyphocephalus* (type, *bisulcatus* Riggenbach, 1898); *Bothridium* (type, *pithonis* Blainville, 1828); *Diplogonoporus*

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(type, *balaenopterae* Lönnberg, 1892); and *Pyramicocephalus* (type, *anthocephalus* Rudolphi, 1810; = *phocarum* Fabricius, 1780 renamed).

Zschokke (1903) described *Dibothriocephalus römeri* from *Trichechus rosmarus* of Arctic seas and transferred the following species from *Bothriocephalus* to *Dibothriocephalus: D. elegans* (Krabbe, 1865) from *Phoca cristata* taken near Godhaven; *D. lanceolatum* (Krabbe, 1865) from *Phoca barbata* taken near Godhaven; *D. polycalceolus* (Ariola, 1896) from *Phoca vitulina;* and *D. quadratus* (v. Linstow, 1892) from *Sternorhynchus* (*Ogmorhinus*) *leptonyx* from south Georgia Islands of the Antarctic Ocean. Another south polar species, *Bothriocephalus antarcticus* Baird, 1853, from *Phoca* sp. was transferred to the genus *Dibothriocephalus* by Shipley (1907).

Meanwhile, Cobbold (1858) had erected the genus *Diphyllobothrium* to contain *D. stemmacephalum* Cobbold, 1858, a cestode from the common porpoise of the North Sea, *Delphinus phocaena,* one of the toothed whales. This genus was not mentioned by Lühe (1899) in his study of the bothriocephalid cestodes.

The classification of the cestodes was revised by Lühe (1910), who arranged the polyzoic forms in four orders: Trypanorhyncha, Pseudophyllidea, Tetraphyllidea and Cyclophyllidea. The Pseudophyllidea were divided into four families: Caryophyllaeidae, Diphyllobothriidae, Ptychobothriidae and Acanthophallidae. The genus *Dibothriocephalus* was regarded as identical with *Diphyllobothrium,* the name was suppressed as a synonym, and the species *D. ditrema* (Creplin, 1825), *D. dendriticum* (Nitzsch, 1824) and *D. fissiceps* (Creplin, 1829), all parasites of birds, were included with *D. latum* in *Diphyllobothrium.* The genus *Bothriocephalus* was included in the family Ptychobothriidae; thus the broad tapeworm of man and the genus to which it was formerly assigned were placed in different families. Nybelin (1922) revised the arrangement of certain pseudophyllidean cestodes, but *Diphyllobothrium* Cobbold was not considered. Although reference was made to Lühe's (1910) paper, Nybelin adopted Lühe's earlier arrangement and the family Dibothriocephalidae. The most comprehensive treatise on the cestodes is the work of Fuhrmann (1931). In the Pseudophyllidea, the latter author recognized seven families; among them, three of the subfamilies of Lühe's were elevated to family rank. The family Diphyllobothriidae was divided into the Ligulinae and Diphyllobothriinae; the latter subfamily contained the genera *Diphyllobothrium* Cobbold (=*Dibothriocephalus* Lühe), *Duthiersia* Perrier, *Bothridium* Blainville (= *Solenophorus* Creplin), *Scyphocephalus* Riggenbach, *Glandicephalus* Fuhrmann, *Lüheella* Baer, *Diplogonoporus* Lönnberg, *Pyramicocephalus* Monticelli, and *Chlamydocephalus* Cohn.

Faust, Campbell and Kellogg (1929) reported eight species of *Diphyllobothrium* from the Far East; they divided the genus into two subgenera: "subgenus *Diphyllobothrium* (e.g., *Diphyllobothrium sensu stricto*), to include those species of the genus *Diphyllobothrium* with rosetted uterine coils and with eggs having rounded ends; and (2) subgenus *Spirometra* n.n., to include those species having a spirally piled outer uterine mass and with eggs having more or less pointed ends. The type species of the subgenus *Diphyllobothrium* is Cobbold's *stemmacephalum* (1858) from *Delphinus phocaena,* which was figured (Cobbold, l. c., *fig.* 80) with a rosetted uterus, while the species *decipiens* is here designated as the type of the subgenus *Spirometra.*" These authors stated, p. 578; "The limited amount of information

available supports the view that species of the subgenus *Diphyllobothrium* require a fish as second intermediate host, in which the sparganum stage develops; and that species of the subgenus *Spirometra* will not develop to the sparganum stage in fishes, but require higher vertebrates, including Amphibia, Reptilia, birds and mammals, for this stage of their life cycle."

Further revision was made by Mueller (1936, 1937). In the former paper he noted further differences between the subgenera of Faust, Campbell and Kellogg and stated: "The writer is of the opinion that when the members of the genus *Diphyllobothrium* are more completely known . . . . it may be necessary to raise these subdivisions to full generic rank." In the latter paper a repartition of the genus *Diphyllobothrium* was presented. Three genera were outlined: (1) unnamed, to include *D. latum* and its relatives. Scolex, small and spatulate; neck, long and slender; outer uterine coils in the form of rosette; cirrus and vagina open in a common sinus; uterine pore separate and posterior; cirrus sac simple; seminal vesicle separate and dorsal to cirrus sac; eggs with rounded ends; coracidium swims with a slow rolling motion; first intermediate host a species of *Diaptomus;* second intermediate host a fish. (2) *Spirometra* n. g., type species, *S. erinacei* (Rudolphi), to include members of the subgenus *Spirometra* of Faust, Campbell and Kellogg. Scolex, small and spatulate; neck, long and slender; anterior uterus in form of a spiral of closely packed coils; cirrus and vagina open separately; cirrus sac composite, enclosing seminal vesicle; eggs pointed; coracidium swims rapidly with hooks to the rear; first intermediate host a species of *Cyclops;* second intermediate host a frog, snake or mammal. (3) Unnamed, to include certain species from seals. Scolex, relatively large, cordate, with bothria extending over anterior proglottids. Neck absent; strobila expanding abruptly from scolex; uterus in form of a rosette; cirrus, vagina and uterine pore open in a common sinus; cirrus sac as in *D. latum;* seminal vesicle separate and dorsal; eggs with rounded ends; life history relationships for the most part unknown. Mueller urged the reexamination of *D. stemmacephalum* and stated: "If *D. stemmacephalum* has characters which definitely set it off generically from *D. latum,* the old name *Dibothriocephalus* will have to be restored for *latum,* which would be an unfortunate, but possibly unavoidable, predicament."

Wardle, McLeod and Stewart (1947) stated that the genus *Diphyllobothrium,* as characterized by Lühe, "is a cumbersome group of about 70 species—many of them of dubious validity—and comprises forms from toothed-whales, seals, sea-lions, carnivorous land mammals and fish-eating birds. Several species have been recorded from humans and one even from a snake.[1] It has always been an unsatisfactory genus to define and analyze, and particularly difficult to evaluate have been the forms from seals and sea-lions that have been recorded by numerous writers." These authors proposed a new genus, *Cordicephalus,* (type, *Taenia phocarum* Fabricius, 1780 from *Phoca barbata,* Greenland), to contain the species found in seals and sea-lions and recognized four species: *Cordicephalus phocarus* (Fabricius, 1780); C. *tectus* (Linstow, 1892); C. *arctocephalinus* (Johnston, 1937); and *C. quadratus* (Linstow, 1892). All other species from seals and sea-lions were re-

[1] *Diphyllobothrium serpentis* Yamaguti, 1935, described from two specimens from the intestine of *Naja Naja atra,* Formosa, may represent an accidental infection; the snake may have eaten the normal host.

garded as identical with one or other of the accepted species. The remaining species of Lühe's genus were distributed among six other genera: *Diphyllobothrium* Cobbold, 1858; *Diplogonoporus* Lönnberg, 1892; *Dibothriocephalus* Lühe, 1899; *Glandicephalus* Fuhrmann, 1921; *Adenocephalus* Nybelin, 1931; and *Spirometra* Mueller, 1937.

Stunkard (1948) described four species of pseudophyllidean cestodes from seals and sea-lions of the North Pacific but, since the descriptions of earlier species are so incomplete and inadequate and since generic concepts are so indefinite and uncertain, taxonomic determinations were not attempted. Data provided by a study of these cestodes contravene the validity of the three generic concepts proposed by Mueller (1937), since in morphology the specimens do not conform to any of the combinations of characters listed by him for the generic groups as outlined. Different combinations of characters occur, and no set of characters seems to be constant. The scoleces of the four species, although variable, may be referred to a common type, short and oval to quadrate to cordiform, but their relation to the anterior end of the strobila is different. In three species the anterior end of the strobila is elongate and narrower than the scolex which is distinctly set off; in the other species the anterior end of the strobila is wider than the scolex. Three species have no neck; one has a short unsegmented neck region. In three of the species, the cirrus, vagina and uterus open into a common atrium; in the fourth there are separate cirral, vaginal and uterine pores. Two species are monogonadic; two are diplogonadic. In all of them the seminal vesicle, "Eschtrichtscher Körper" or "Propulsionsblase" as the organ has been variously designated, is external to the cirrus sac, and the eggs have rounded ends.

The report by Mueller (1937) that *D. lanceolatum* has glandular cells in the scolex and the finding of such cells in the material from seals and sea-lions of the North Pacific may impugn the validity of *Glandicephalus* Fuhrmann, 1921 and probably also of *Adenocephalus* Nybelin, 1931. As pointed out by Stunkard (1948), *Taenia phocarum* Fabricius, named by Wardle, McLeod and Stewart (1947) as type of the proposed new genus *Cordicephalus,* had been named as type of the genus *Pyramicocephalus* by Monticelli (1890). The genus *Diphyllobothrium,* as at present constituted, is a heterogeneous collection of species, but the features on which valid generic concepts can be erected are not yet clear.

The first, most obvious and most desirable determination is whether or not the broad tapeworm of man, *D. latum,* is congeneric with *D. stemmacephalum.* The description of Cobbold is brief, incomplete, and the only redescription is a doctoral dissertation by Cohn (1912) done under the supervision of Prof. Max Braun and not widely distributed. Cohn redescribed *D. stemmacephalum* from three specimens: (1) an incomplete worm, 1.5 meters long with about 1400 proglottids, lacking scolex and anterior proglottids but with terminal proglottid, sent by Cobbold to Leuckart and loaned by Prof. Spengel of the zool. Institut, Giessen; (2) an incomplete worm, 1.45 meters long, lacking scolex and neck and terminal proglottid, much contracted and not well preserved, collected by K. E. von Baer from the intestine of the common porpoise taken near Königsberg; (3) several pieces, probably of a single specimen since only one scolex and one terminal proglottid were present, collected 1905 from the same host and locality. The material was well preserved and the specific description, with morphological details from serial sec-

tions, is based largely on it. Both of the latter specimens were from the zoological museum, Königsberg. Cohn stated that the three specimens undoubtedly were members of the same species. He listed differences between *D. stemmacephalum* and the broad tapeworm of man, which in his opinion are so pronounced that the two species can not be regarded as congeneric. The parasite of man, however, has been designated as *Diphyllobothrium latum* in subsequent literature.

MATERIAL AND METHODS

Through the kindness of Prof. J. J. C. Buckley, of the London School of Hygiene and Tropical Medicine, I received on loan two slides, one with a scolex and anterior proglottids and the other with eight gravid proglottids of *D. stemmacephalum,* both prepared by Cobbold and so far as can be determined, the only material of the species now available in England. The worms were dead and apparently partially decomposed when collected. The specimens were not stained and fixation, if attempted, was poor. The structure of the scolex and adjacent proglottids can be determined, but little could be learned concerning the morphology of the gravid proglottids. Examination by the use of polarized light and by the phase microscope was not helpful; the genital and uterine pores were obscured by eggs in the terminal part of the uterus. So permission was sought and obtained to unmount the gravid proglottids in an attempt at fixation and staining. Photographs and camera lucida tracings were made of the proglottids, they were then unmounted and slowly transferred to water. After a period of softening they were soaked in a 0.25% solution of trisodium phosphate, washed in water, fixed in Zenker's fluid, washed in water, treated with iodine in alcohol, and stained with Ehrlich's acid haematoxylin. Two proglottids were removed, embedded in paraffin and cut in transverse serial sections at a thickness of 15 microns. The remaining proglottids were differentiated, dehydrated, cleared and mounted. During the dehydration and clearing the tissues shrank somewhat, the coils of the uterus were crowded together and are less distinct, and the lateral edges of the proglottids tended to curve ventrally. The staining made it possible to recognize the testes, ovary and vitellaria, and the sections provided information concerning the copulatory organs, as well as the genital and uterine pores.

DESCRIPTION

The scolex (Fig. 1, 3) is oval in lateral view, 0.3 mm long and about 0.44 mm in depth. It is turned about 80 degrees with respect to the strobila, so a flattened lateral aspect is presented. This condition usually obtains in mounted specimens of diphyllobothrid cestodes unless the coverglass is supported or the specimen has been well fixed and sufficiently hardened to withstand the pressure of the coverglass. The dorsal and ventral grooves are clearly visible and extend the entire length of the scolex. It has not been possible to measure the width exactly, but calculations of the depth of focus from one side to the other indicate that it is about one-third as wide as long. The scolex is distinctly set off both dorsoventrally and laterally from the strobila. As noted, the neck is turned, presenting a side view which measures 0.19 mm in thickness. In this portion there are faint transverse striations but no proglottid formation. About 0.3 mm behind the scolex, the first furrows suggest the beginnings of strobilation, and the width of the worm gradually increases posteriorly. The portion on the slide (Fig. 1) measures 7.94 mm in length and contains 264 recognizable proglottids. They increase in size and distinctness posteriorly and the terminal two-fifths of the strobila contains 52 proglottids; the 264th proglottid is 0.07 mm long and 2.14 mm wide. There are no visible cellular aggregates to indicate the rudiments of reproductive organs. In this portion of the strobila there are faint longitudinal lines, indicated in Fig. 1, but they could not be traced into the scolex and because of the condition of the specimen it was impossible to determine whether they represent longitudinal furrows on the surface or excretory tubules; possibly both are represented.

The gravid proglottids (Fig. 5) are craspedote, but the lateral margins are not markedly serrate. The segments measure about 4.25 mm in length and 11–12 mm in width. The thickness varies between 0.2 and 0.3 mm, except in the region of the uterus. The muscle layers characteristic of diphyllobothrid cestodes are present but the severe distortion and rupture of tissues render precise description impossible and measurements may not be significant. The medullary portion of the proglottids is relatively thin, about one-fourth the total thickness. Excretory tubules and nerve trunks could not be traced with certainty.

Explanation of Figures

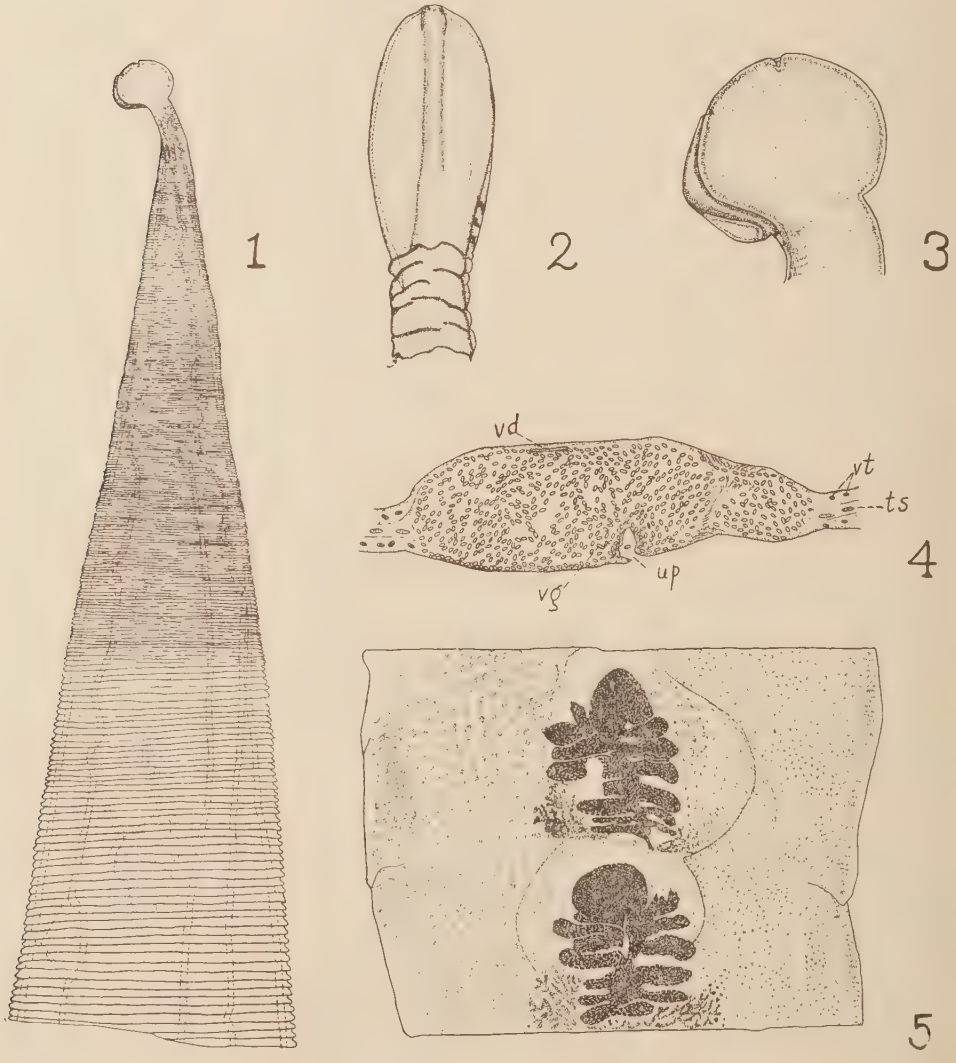

Fig. 1. *D. stemmacephalum,* type specimen, scolex and anterior proglottids.
Fig. 2. *D. latum,* scolex, drawn at the same magnification as Fig. 1.
Fig. 3. *D. stemmacephalum,* scolex, same as Fig. 1, enlarged.
Fig. 4. *D. stemmacephalum,* cross section of the median field, through the uterine pore.
Fig. 5. *D. stemmacephalum,* two proglottids; the gap, on the left side near the middle of the uterus of the anterior proglottid, is an artifact; testes on left, vitellaria on right.

Abbreviations

ts, testis; up, uterine pore; vd, vas deferens; vg, vagina; vt, vitellaria

There is a shallow genital atrium into which the cirrus and vagina open; the cirrus immediately in front of the vagina. The atrium is median, situated about 0.6 mm from the anterior end of the proglottid and obscured in whole mounts by loops of the uterus which extend anteriorly on both sides and also lie above it. The uterine pore is median, about 0.9 mm behind the common genital sinus. These distances were calculated by counting the number of serial sections and multiplying by their thickness.

The cirrus sac extends anteriorly and dorsally from the genital atrium; it is pyriform, about 0.3 mm in length and 0.2 mm in greatest width, and contains a coiled, sperm-filled duct. Dorsally and posteriorly it communicates with an oval, thick-walled, muscular, seminal vesicle or "Propulsionsblase", almost as large as the cirrus sac. Muscle fibers form continuous sheets in the walls of the two structures. From the vesicle, the vas deferens extends posteriad in wide loops on the dorsal side of the body; it was traced to the level of the anterior margin of the ovary. The testes seem to be interrupted, at least partially, at the interproglottidal areas. There are 15–27 in a transverse section on each side of the body and 20–30 in a longitudinal section, about 1000 in each proglottid. The follicles are oval, 0.09–0.16 mm in diameter. They are arranged in a single layer, except in the lateral portions of the strobila where they overlap each other. Follicles of the two sides do not meet in the median plane, as the most anterior loop of the uterus in each proglottid is almost contiguous with the ovary of the preceding one. They encroach at the sides of the uterine and ovarian field.

The vagina passes posteriad from the genital sinus, on the ventral side of the proglottid, winding slightly between the uterine coils. It could be identified in sections by the presence of spermatozoa, but could not be traced to the seminal receptacle, located behind the isthmus of the ovary. The ovary is a relatively large, two-winged organ connected medially by the isthmus. It has a reticulate appearance, is approximately as wide as the uterine field, about 2.5 mm, and the lateral portions extend forward about one-fourth of the length of the proglottid. The seminal receptacle, vitelline receptacle, Mehlis' gland and the initial loop of the uterus are immediately posterior and dorsal to the isthmus of the ovary but the course of the ducts could not be traced. Mehlis' gland is relatively small and the cells did not stain well. In the initial loops of the uterus, which lie behind and above the ovary, the egg shells are thin and the contained cells are stained, in the next 2–4 loops on each side of the body the eggs have thin shells, while the terminal loops are superposed and congested with enormous numbers of thick-shelled eggs. At the level of the ovary the median field of the proglottid is only slightly, if any, thicker than the adjacent portions, 0.2–0.3 mm, but anteriorly, as the uterus fills with eggs, this portion of the proglottid becomes increasingly thicker and at the uterine pore (Fig. 4) the median field is 0.9 mm in thickness. As shown in the figure, the cortical parenchyma is so reduced that only a thin strand of tissue intervenes between the eggs and the cuticular covering of the proglottid. There are more than 900 eggs in the section shown in Fig. 4 and the massing of eggs in the distal two-thirds of the uterus renders this portion of the proglottid entirely opaque. The anterior coils of the uterus extend forward on both sides of the cirrus sac and in the whole mount they cover it entirely. Study of sections suggests, however, that this last condition is an artifact and that the uterine coils normally do not cover the cirrus sac although they do overlie the common genital sinus. The vitellaria occupy the cortical areas, dorsally and ventrally, except in the uterine field and in the velar projections. They are continuous from one proglottid to the next, with no interproglottidal interruption, but do not meet in the median plane.The follicles measure 0.05–0.075 mm in diameter; collecting ducts pass mediad on either side at the ovarian level and meet to form the vitelline receptacle. The eggs are oval, operculate, 65–70 microns long and 45–50 microns wide.

DISCUSSION

Study of the type material of *Diphyllobothrium stemmacephalum* supplements the original description of Cobbold and that of Cohn (1912). According to Cobbold, the worms measure up to 10 feet in length and mature proglottids are one-twelfth to one-sixth of an inch deep by one-half inch in breadth. His reference to depth must indicate length of proglottids, which is about one-third the width.

In a cotype specimen, sent by Cobbold to Leuckart, which lacked the scolex and anterior proglottids and which measured 1.5 meters long, Cohn counted 1400 proglottids. This specimen must have been almost complete, since the anterior proglottids were smaller than the terminal ones shown in Fig. 1. The region before the first gravid proglottid was 8.5 cm long and contained about 550 proglottids. The

proglottids became progressively longer posteriorly and the width of the strobila increased in the first half of the body to 13 mm, and gradually diminished posteriorly. Cobbold reported 10–12 longitudinal furrows on the surface of the strobila and Cohn found 10 such furrows on the specimen sent by Cobbold to Leuckart. But Cohn's Fig. 4, showing a superficial view of a proglottid from his third specimen, which was fixed alive and well preserved, suggests that the supposed furrows are actually tubules of the excretory system in the cortical parenchyma. This specimen was more extended, elongate and narrower than the other two, but otherwise the three specimens studied by him were similar. In the extended specimen, the genital primordia were visible about 10 cm from the scolex, the anlagen of the cirrus sacs at 25 cm, the anlagen of the vitellaria at 30 cm, the first eggs at 38 cm, and fully gravid proglottids about 53 cm from the scolex. In general organization, the strobila of *D. stemmacephalum* is very similar to that of *D. latum*.

The present study confirms the account of Cohn with but one exception; he stated that in the Cobbold specimen each gravid proglottid has a midventral protuberance, caused by the protrusion of the cirrus sac, with separate male and vaginal pores on the posterior side of the protuberance. Cobbold had reported conspicuous reproductive orifices in the mesial line, but gave no details. From a study of serial sections, Cohn did not describe the relation of the two genital pores, but stated that the vagina runs dorsally along the posterior wall of the cirrus sac, turns ventrad in front of the seminal vesicle, and continues posteriad on the ventral side of the body. On p. 28 he referred to: "Papillen, die ein ovales Feld um Cirrus- und Vaginalöffnung herum bilden." It is apparent that the two openings are very close to each other and if the cirrus sac were protruded ventrally it might obliterate the shallow, atrium, observed in the present study, and the two pores would appear one behind the other on the surface of the proglottid.

The greatest difference between *D. stemmacephalum* and *D. latum* is in the shape and relative size of the scolex. The scolex of *D. stemmacephalum* is portrayed in the figures by Cobbold, by Cohn, and in Fig. 1. In the specimen from *Delphinus dussumieri* identified by Yamaguti (1935) as *D. stemmacephalum,* the scolex is short, distorted, but similar in shape to the one figured by Cohn, with the bothria coalesced at the apex and divergent posteriorly, whereas in the Cohn specimen the bothria are overlapped posteriorly and divergent anteriorly. It is apparent that the scolex is a very mobile organ, capable of great changes in form. It is very small, shorter than deep, and distinctly set off from the anterior end of the strobila. In lateral view it may vary from oval to cordiform to triangular; in no case however does it manifest the elongate, spatulate form of the scolex of *D. latum*. Comparison of Fig. 1 and 2, showing scoleces of *D. stemmacephalum* and *D. latum* drawn at the same magnification, demonstrates the essential differences in shape and relative size. Serial sections of the scolex of *D. stemmacephalum* are not available and consequently histological differences, if present, have not been observed. *D. latum* is a larger species, with greater length as well as size of scolex and proglottids, but the form of the scolex appears to be distinctive.

Mature proglottids of *D. stemmacephalum* differ from those of *D. latum* in the course of the uterus. According to Cohn, the uterus is approximately the same length in the two species but in *D. stemmacephalum* there are 12–15 loops on each side of the median plane whereas in *D. latum* there are only 5–7 such loops. Fur-

thermore, in the former species Cohn reported that the loops are arranged in an alternating, "zickzack" sequence and do not cross each other to form a "rosette" pattern. The figures of Cohn obviously were made from relatively young proglottids and at that stage, before the coils become congested with eggs, the uterine pattern can be determined accurately. In fully gravid proglottids it is not possible to follow the course with certainty and, especially in the terminal portion, the loops may be superposed. In the Cobbold specimen (Fig. 5), 6–9 uterine loops can be recognized and possibly others are present but not visible. Comparison with *D. latum,* where material is abundant, shows in that species young proglottids 1.1 mm long and 4.8 mm wide have no eggs in the uterus. Here the canal winds forward irregularly, with 5–8 lateral sinuosities. In slightly older proglottids, 1.63 mm long and 5.88 mm wide, the uterus is filled with eggs; the first three loops on each side contain thin-shelled eggs and the terminal 3–4 loops are congested with thick-shelled, yellow eggs. In development, the course of the uterus changes from a "spiral" to a "rosette" form and this change is concomitant with and undoubtedly results from the amassing of eggs in the organ. Although the uterus of *D. latum* manifests much variation, it never develops as many loops as are shown in Cohn's Figs. 4 and 5 of *D. stemmacephalum.*

Cohn reported that in *D. stemmacephalum* the ovary is relatively smaller than in *D. latum;* that laterally it is confined to the midfield, whereas the ovary of *D. latum* extends into the lateral areas.

Differences in the shape and relative size of the scolex, in the course of the uterus, and extent of the ovary constitute the major features in which *D. stemmacephalum* differs from *D. latum.* Other differences were noted by Cohn, but they appear to be of minor significance. Authorities disagree concerning the features which characterize genera of the pseudophyllidean cestodes. Certain authors regard the scolex as of primary importance whereas others consider it a secondary structure, merely a mobile organ of attachment, and ascribe greater importance to the musculature and morphology of the reproductive organs. Strong arguments may be presented to support both points of view and each has its own inherent weaknesses. The cestode orders are characterized primarily by the structure of the scolex; in the infective larvae the scolex, except for size, is virtually identical with that of the adult stage; the pseudophyllidean families may be distinguished by the form of the organ, and it is natural to expect that the scoleces of diphyllobothrid genera would be distinctive. The differences between the scoleces of *D. stemmacephalum* and *D. latum* are shown in Fig. 1 and 2. The genital orifices and copulatory organs of the two species are very similar, but there are differences in the shape of the ovary and the course of the uterus. So in both scoleces and proglottids, these species manifest morphological differences, but whether these differences are to be regarded as generic or merely specific is yet controversial.

Moreover, taxonomic determinations are based on life cycles and host relations as well as on anatomical features. Mueller (1937) stated: "It seems rather improbable that the same genus of cestode will be found in both the porpoise and land-dwelling mammals." *Diphyllobothrium latum* requires fresh-water crustaceans and fishes as intermediate hosts, whereas the corresponding hosts of *D. stemmacephalum* are presumably marine organisms. This circumstance focuses attention on the bionomic problem of related species in marine and fresh-water hosts. The subject, as

it pertains to digenetic trematodes, was discussed by Stunkard (1930) and Stunkard and Shaw (1931). The physical, chemical and biotic factors which permit or prevent completion of trematode life histories must similarly affect the diphyllobothrid cestodes, although in the latter group only one larval stage, the coracidium, is free-living. These larval stages are very delicate, ephemeral, and incapable of any extended migration. They must find their next hosts in the immediate vicinity and consequently there is a distinct separation of marine and fresh-water faunas. It is commonly accepted that the toothed whales date from the early Miocene epoch and although certain of them ascend rivers, the group is essentially marine. The terrestrial and marine hosts of diphyllobothrid cestodes have long been distinct and it is questionable if they harbor cestodes of the same genus.

Furthermore, the host relations of cyclophyllidean cestodes are narrowly restricted. Discussing this subject, Baer (1946) wrote: "Les Cestodes adultes sont étroitement adaptés a leurs hôtes et cette spécificité est d'autant plus marquée que ceux-ci appartiennent à des groupes de Vertébrés supérieurs." Referring specifically to the tapeworms of mammals he stated that they, "présentent une spécificité plus marquée, puisque chaque ordre possède sa faune caractéristique. Chez les Cestodes comme chez les Mallophages et les Puces, la classification des parasites se superpose à celle des hôtes, les formes les plus anciennes s'observant chez les hôtes les plus primitifs au point que la répartition de ces Vers chez leurs hôtes ne doit pas être rapportée à des causes éthologiques mais bien plus aux affinitiés zoologiques des hôtes entre eux." If the same principles can be applied to pseudophyllidean species, the argument for generic separation of *D. stemmacephalum* and *D. latum* would receive strong support.

Diphyllobothrium latum has been reported from a large number of hosts, including man, several species of wild and domestic cats and dogs, the mongoose, the walrus, various seals and sea-lions, bears, foxes, the mink and the domestic pig. But it is certain that many of these records do not pertain to *D. latum* and it is questionable how many species have been confused in these reports or what animals may actually serve as hosts of *D. latum*. Magath (1929) described specimens from a dog which had been fed plerocercoids from lake fish at Ely, Minnesota and concluded that *D. parvum* (Stephens, 1908) was only a young stage of *D. latum*. His Fig. 7 shows terminal segments longer than wide with at least 9 or 10 uterine loops on each side of the median plane. He reported that these terminal segments are shed within a week, after which the worm takes on the characteristics of the typical *D. latum* with its broad segments. He stated: ". . . the shedding of the terminal segments is associated with the morphologic change in the worm." Scott (1932) reported that the diphyllobothrid cestodes of bears in Yellowstone Park are *D. cordatum* (Leuckart, 1863) and that the eggs and plerocercoids of *D. cordatum* are indistinguishable from those of *D. latum*. So far as I am aware, there have been no complete and controlled studies, such as the one by Venard (1938), in which onchospheres from a known strobila of *D. latum* have been carried to sexual maturity in experimental hosts. The extent of variation which may occur normally or may be induced by development in unusual hosts must be known before specific limits of *D. latum* can be determined. No species of *Diphyllobothrium* from mammals can be positively and fully characterized at the present time, and until this problem is clarified, further taxonomic work on species of the genus is more or less pointless.

Comparison of *D. stemmacephalum* and *D. latum* might readily lead to the conclusion that the two species are members of different genera. Indeed Cobbold, who was familiar with *D. latum,* obviously did not regard the two species as congeneric, and he placed *Diphyllobothrium* between *Bothriocephalus* and *Echinobothrium.* But studies of other diphyllobothrid species, especially those from pinnipeds, have yielded data which complicate the situation and render determination of the taxonomic relations of these species very difficult. Fuhrmann (1920) accepted Cohn's opinion that *D. stemmacephalum* and *D. latum* belong to different genera, and he included the several species of pseudophyllidean cestodes from antarctic pinnipeds in the genus *Dibothriocephalus.* His figures, particularly of *D. perfoliatus* (Railliet and Henry, 1912) and *D. mobilis* Rennie and Reid, 1912, show that the scoleces are exceedingly variable in shape and that they may assume the elongate, spatulate form characteristic of *D. latum* or the short, oval or truncated shape of *D. stemmacephalum.*

Baer (1924, 1925) described a species from *Otocyon megalotis* in the Sir Arnold Theiler collection of South African cestodes as *Lüheella pretoriensis* n.g., n.sp., and erected a new family LÜHEELLIDAE to contain it. The generic diagnosis of *Lüheella* given by Baer is virtually identical with that of *Spirometra* as given by Faust, Campbell and Kellogg (1929) and by Mueller (1937); consequently *Spirometra* appears to be a subjective synonym of *Lüheella,* since different species were selected as types of the two genera. But Baer and Joyeux, in a footnote in Joyeux and Houdemer (1928), stated that the genus *Lüheella* and family LÜHEELLIDAE were based on the absence of an external seminal vesicle, a character regarded as diagnostic of the family DIPHYLLOBOTHRIIDAE. They reported: "L'étude de la poche du cirre chez *D. mansoni, D. raillieti* and *D. theileri* nous a montré tous les types intermédiaires entre celle de *D. latum* d'une part et de *L. pretoriensis* d'autre part." Accordingly, the family LÜHEELLIDAE and the genus *Lüheella* were relegated to synonymy with DIPHYLLOBOTHRIIDAE and *Diphyllobothrium* respectively, and the type species of *Lüheella* was designated *Diphyllobothrium pretoriense* (Baer, 1924).

From his studies on variation in the shape of the scolex, and on the observations of Baer and Joyeux that all intermediate conditions may occur between separation of the seminal vesicle and cirrus sac and inclusion of the vesicle within the sac, Fuhrmann (1931) listed *Dibothriocephalus* as a synonym of *Diphyllobothrium.* In four species of diphyllobothrid cestodes from Alaskan pinnipeds, Stunkard (1948) found different combinations of characters and no set of characters appears to be constant or invariably associated. He showed that the proposal by Zschokke (1903) to base classification on the arrangement of the excretory tubules was impracticable, since the inordinate variation in their distribution precluded their use for taxonomic purposes. He showed further that the bases proposed by Mueller (1937) and by Wardle, McLeod and Stewart (1947) were inadequate for subdivision of the genus *Diphyllobothrium.*

Restudy of the limited material of *D. stemmacephalum* has not provided data on which valid generic concepts can be formulated within the limits of the present genus *Diphyllobothrium.* Species now included in the genus constitute a heterogeneous collection, from a variety of hosts and bionomic areas, but the morphological diversity is so distributed among the species and so many different combinations of characters exist that arrangement of the species into related groups must await further information.

ABSTRACT-SUMMARY

The taxonomic history of the broad tapeworm of man, *Diphyllobothrium latum,* is traced. Its relation to *D. stemmacephalum* Cobbold, 1858, type of the genus, is discussed. Type material of the latter species has been restudied and compared with other diphyllobothrid species. Although the genus *Diphyllobothrium* as now constituted comprises a large and heterogeneous collection of species, the features on which valid generic concepts can be formulated within the group are not yet clear.

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CHANDLER, ASA C. (M.S., Ph.D. Prof. Biology, Rice Institute, Houston, Texas. Former Officer-in-Charge, Hookworm Research Laboratory, School Tropical Medicine and Hygiene, Calcutta, India). INTRODUCTION TO PARASITOLOGY, 8th ed. v + 756 pp. 28 chapters, 273 text-figures, 22 pp. index. Copyright 1949. John Wiley & Sons, Inc., New York. $6.00.

CHANG, K. AND CO-WORKERS. STUDIES ON HOOKWORM DISEASE IN SZECHWAN PROVINCE, WEST CHINA. v + 152 pp., 41 tables, 34 text-figures, 4 pp. references. Copyright 1949. The Johns Hopkins Press. $3.00.

CLEVELAND, L. R. (Prof. Biology, Harvard University). THE WHOLE LIFE CYCLE OF CHROMOSOMES AND THEIR COILING SYSTEMS, New Series, Vol. 39, Part 1, 1949. (Transactions of the American Philosophical Soc.) 100 pp. 2 tables, 35 plates, 93 text-figures, 3 pp. index. The American Philosophical Society, Phila. $1.50.

FAUST, ERNEST CARROLL (A.B., M.A., Ph.D. The William Vincent Professor of Tropical Diseases and Hygiene, Head Division of Parasitology, Dept. Tropical Medicine and Public Health, The Tulane University of Louisiana, New Orleans.) HUMAN HELMINTHOLOGY, 3rd ed., 744 pp. 7 sections, 37 chapters, 313 text-figures, 26 pp. subject index, 10 pp. author's index. Copyright 1949. Lea & Febiger, Phila. $10.00.

HORSFALL, FRANK L., JR. (Hospital of the Rockefeller Institute for Medical Research.) DIAGNOSIS OF VIRAL AND RICKETTSIAL INFECTIONS. Symposium held at the New York Academy of Medicine, Jan. 29 and 30, 1948. Section on Microbiology, 153 pp., 12 chapters, 7 text-figures, 11 tables, 24 pp. references. Copyright 1949. Columbia University Press, New York. $3.75

MACLEOD, COLIN M. (M.D.) Editor. EVALUATION OF CHEMOTHERAPEUTIC AGENTS. Symposium Number Two, Symposia of the Section of Microbiology, The New York Academy of Medicine. vii + 205 pp. 14 chapters, 15 text-figures, 14 tables, 33 pp. references. Copyright 1949. Columbia University Press, New York. $4.00.

THOMAS, EVAN W. (M.D. Prof. Clinical Medicine, New York University College of Medicine; Director Rapid Treatment Center and Visiting Physician, Bellevue Hospital, New York; Consultant, U.S. Public Health Service.) SYPHILIS: Its Course and Management. v + 317 pp. 14 chapters, 68 text-figures, 15 pp. index. Copyright 1949. The Macmillan Co., New York. $5.50.

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(No. 6, Section 2)

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The Journal of Parasitology

Vol. 35, No. 6, Section 2 (Supplement) December, 1949

PROGRAM AND ABSTRACTS OF THE TWENTY-FOURTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

NEW YORK, NEW YORK

December 27, 28, 29, 1949

PROGRAM[1]

TUESDAY MORNING SESSION, DECEMBER 27, 9:00 AM, HOTEL STATLER, GEORGIAN ROOM.

Read

1. Phosphorus Deficiency, a Limiting Factor in Fowl Parasitism. (8 min) (Lantern) J. E. ACKERT AND S. M. GAAFAR, Kansas State College, Manhattan.

2. Lethal Effects of Acetic Acid on Larvae of *Ancylostoma caninum* in Fecal-Soil Cultures. (7 min) (Lantern) J. E. ACKERT AND F. L. LIGENZOWSKI, Kansas State College, Manhattan.

3. A New Species of the Free-Living Nematode Genus *Rhabditis* of Interest in Comparative Physiology and Genetics. (10 min) (Lantern) ELLSWORTH C. DOUGHERTY, University of California, Berkeley, AND VICTOR NIGON, Faculté des Sciences P.C.B., Paris.

4. Some Abnormal Host Relationships of *Nippostrongylus muris*. (15 min) (Lantern) WILLIAM D. LINDQUIST, Michigan State College, East Lansing.

5. Results of Feeding Small Amounts of Phenothiazine in Pure Infections of the Nodular Worm (*Oesophagostomum radiatum*) in the Calf. (15 min) (Lantern) ROY L. MAYHEW, Louisiana State University.

6. Thyroid Abnormalities as a Possible Factor in Trichostrongylidosis. (15 min) (Lantern) JOHN H. WHITLOCK, New York State Veterinary College.

7. Preliminary Observations on the Life History of *Ascaris columnaris*. (10 min) (Lantern) JACK D. TINER, University of Illinois.

8. Observations on the Pathogenicity of *Nematodirus spathiger* in Lambs. (10 min) (Lantern) K. C. KATES AND J. H. TURNER, U. S. Bureau of Animal Industry.

9. A Nephelometric Method of Calibrating the Photo-Electric Light Meter for Making Egg-Counts by Direct Fecal Smear. (15 min) PAUL C. BEAVER, The Tulane University of Louisiana.

10. Effect of Purified Diets on the Host-Parasite Relationship of Chickens to *Ascaridia galli*. (15 min) (Lantern) ELVIO H. SADUN, JOHN R. TOTTER AND CECILIA K. KEITH, University of Arkansas.

[1] An alphabetical author index will be found at the end of the program. Extra copies of this supplement, and portraits of parasitologists, will be on sale at the meeting.

11. The Cultivation of the Free-Living Stages of Parasitic Nematodes in the Absence of Living Bacteria. (15 min) (Lantern) PAUL P. WEINSTEIN, Johns Hopkins School of Hygiene. (Now with National Institutes of Health)

12. Immunity to Reinfection in Trichinosis. (15 min) (Lantern) IRVING RAPPAPORT, Long Island College of Medicine, AND HELEN S. WELLS, School of Public Health, Columbia University.

TUESDAY AFTERNOON SESSION, DECEMBER 27, 2:00 PM, HOTEL STATLER, GEORGIAN ROOM.

Read

13. Effect of Low Temperatures on Acquisition of Parasites by Swine. (10 min) (Lantern) JOHN S. ANDREWS, U. S. Bureau of Animal Industry, Tifton, Georgia.

14. Giant Kidney Worm, *Dioctophyma renale*, in a Dog. (15 min) (Lantern, 2 × 2 Kodachrome) GEORGE L. GRAHAM AND JAMES H. MARK, School of Veterinary Medicine, University of Pennsylvania.

15. Evidences of Acquired Immunity in the Cotton Rat to Infection with the Filarial Worm, *Litomosoides carini*. (15 min) (Lantern) ETTA MAE MACDONALD AND J. ALLEN SCOTT, University of Texas Medical Branch, Galveston.

16. Intestinal Flagellates of a Wallaroo, *Macropus robustus*. (10 min) (Lantern) HAROLD KIRBY AND BRONISLAW HONIGBERG, University of California.

17. A Contribution to the Discussion about the Relationship of *Chilomastix* and *Retortamonas*. (5 min) (Lantern) HAROLD KIRBY AND BRONISLAW HONIGBERG, University of California.

18. Some New Chemotherapeutic Agents in Experimental Enterohepatitis (Blackhead) of Turkeys. (15 min) (Lantern) E. WALETZKY, M. C. BRANDT, A. BLIZNICK AND C. O. HUGHES, Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company.

19. The Development of Resistance to and the Effect of Some New Chemotherapeutic Agents on Enterohepatitis Induced by the Oral Administration of Cecal Worm Ova to Chickens and Turkeys. (15 min) (Lantern) STERLING BRACKETT AND ALEXANDER BLIZNICK, Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company.

20. Observations of *Endamoeba histolytica* in Microcultures of *Trichomonas foetus*. (15 min) (Lantern) CHARLES W. REES, National Institutes of Health.

21. A Highly Efficient Medium for the Cultivation of *Endamoeba histolytica*, Suitable for Drug Testing. (15 min) (Lantern) ERNEST HARTMAN, Stritch School of Medicine, Loyola University, Chicago.

22. Some Observations on Vitamins and Antibiotics in the Cultivation of *Endamoeba histolytica*. (10 min) E. CLIFFORD NELSON, Medical College of Virginia.

23. A Stain for the Rapid Differentiation of the Trophozoites of the Intestinal Amoebae in Fresh, Wet Preparations. (8 min) (Lantern, 2 × 2 Kodachrome) (also by demonstration) CLARENCE A. VELAT, PAUL P. WEINSTEIN AND GILBERT F. OTTO, The Johns Hopkins University.

24. Observations on the Occurrence of *Rickettsia tsutsugamushi* in Rats and Mites in the Malayan Jungle. (15 min) (Lantern, 2 × 2 Kodachrome) ROBERT

Traub, Lyman P. Frick and Fred H. Diercks, Army Medical Department Research and Graduate School, Washington, D. C.

Tuesday Evening, December 27, 7:00 PM, Hotel Statler, Parlor A
Dinner and business meeting, officers and members of the Council.

Wednesday Morning Session, December 28, 9:00 AM, Hotel Martinique, Gold Room.

Symposium

The Physiology of Parasites

25. Oxygen Requirements of Parasites. (30 min) James W. Moulder, The University of Chicago.

26. Effect of Drugs on Metabolism and Enzyme Systems of Parasites. (30 min) (Lantern) Ernest Bueding, Western Reserve University.

27. The Carbohydrate Metabolism of Parasites. (30 min) (Lantern) Theodor von Brand, National Institutes of Health.

28. Protein Metabolism of Parasites. (30 min) (Lantern) Quentin M. Geiman and Ralph W. McKee, Harvard School of Public Health.

Presidential Address

29. Osler and Parasitology. Thomas W. M. Cameron, Macdonald College, Quebec.

Wednesday, December 28, Hotel Statler, Grand Ballroom.
1:30 PM, Annual Luncheon and Business Meeting.

Wednesday Afternoon Session, December 28, 2:30 PM, Columbia University, Schermerhorn Hall, Morningside Heights.

By Demonstration

23. A Stain for the Rapid Differentiation of the Trophozoites of the Intestinal Amoebae in Fresh, Wet Preparations. (Also read) Clarence A. Velat, Paul P. Weinstein and Gilbert F. Otto, The Johns Hopkins University.

30. The Effects of X-Ray on *Rhabditis* Sp. Lyell J. Thomas and Henry Quastler, University of Illinois.

31. Strain Differences in *Plasmodium gallinaceum* Brumpt. Joseph Greenberg, Helen Louise Trembley and G. Robert Coatney, National Institutes of Health.

33. The Occurrence of an Adult Holostome (Trematoda: Cyathocotylidae) in the Intestine of a Fish. R. M. Cable and Winona B. Vernberg, Purdue University.

34. Mucoid Glands in *Fasciola hepatica* Cercariae. Francis J. Kruidenier, University of Illinois.

35. *Eimeria meleagrimitis* Tyzzer, 1929 in the Turkey. Phillip A. Hawkins, Michigan State College.

36. Echinococcosis in Lebanon and its Incidence in Animal Hosts. Alan C. Pipkin, University of Arkansas Medical School; Emile Rizk and Jirair Balikian, American University Medical School, Beirut.

37. A Natural Mammalian Final Host (*Sorex* sp.) for an Acuariid Nematode. JACK D. TINER, University of Illinois, AND ROBERT RAUSCH, U. S. Public Health Service, Anchorage, Alaska.

38. The Food of *Cyathostomum* (Nematoda: Strongylidae) Adults. NORMAN D. LEVINE, University of Illinois.

39. Sporocyst Generations of *Postharmostomum laruei* McIntosh (Trematoda: Brachylaemidae). MARTIN J. ULMER, University of Michigan.

40. An Anomalous Tapeworm Strobila Showing Reversal of the Proglottids. KATHEL B. KERR, Dr. Salsbury's Laboratories, Charles City, Iowa.

41. The Eyelid Lesion Appearing in Chicks Infected with *Plasmodium lophurae*. ELERY R. BECKER, Iowa State College.

42. Dicrocoelid Trematodes from the Gorilla. HORACE W. STUNKARD, New York University.

43. Observations with the Phase Microscope on the Malaria Parasite *Plasmodium lophurae* and on its Extracellular Development *in vitro*. WILLIAM TRAGER, Rockefeller Institute, Princeton.

44. Infection of the Immature White Mouse with the Avian Parasite, *Plasmodium lophurae*. (Also read) R. BARCLAY MCGHEE (Introduced by William Trager), Rockefeller Institute, Princeton.

45. The Mouth Parts of *Liponyssus bacoti* Hirst. (Also read) FLORA E. GORIROSSI, The University of Texas, Medical Branch, Galveston.

109. The Life Cycle of *Diphyllobothrium latum*. (15 min) (Motion Picture) M. S. FERGUSON, Communicable Disease Center, Atlanta.

110. Ultra-rapid Demonstration of Blood Parasites by Thedane Blue Methods. (10 min) (Motion Picture) H. C. R. SIMONS, Chemical Biological Center, National Research Council, Washington, D. C.

111. The Life of Dr. Howard Taylor Ricketts. (Scrapbook) W. MALCOLM REID, Monmouth College, Monmouth, Illinois.

THURSDAY MORNING SESSION, DECEMBER 29, 9:00 AM, COLUMBIA UNIVERSITY, SCHERMERHORN HALL, MORNINGSIDE HEIGHTS, ROOM 501.

Read

46. Proof of the Direct Action of an Antibiotic on *Entamoeba histolytica*. (15 min) (Lantern) WILLIAM BALAMUTH AND MORGAN M. BRENT, Northwestern University.

47. Experimental Infection of Guinea Pigs with *Endamoeba histolytica*. (15 min) (Lantern) D. JANE TAYLOR, JOSEPH GREENBERG AND G. ROBERT COATNEY, National Institutes of Health.

48. Further Studies on the Factor that Spares Erythrocytes from Phagocytosis and its Possible Significance in Malarial Infection. (15 min) (Lantern) ELERY R. BECKER, ALICE A. MAROUSEK, DORWIN BYRD AND THOMAS SCHWINK, Iowa State College.

49. The Antimalarial Activity of the Plasma of Certain Adult Ducks against *Plasmodium lophurae*. (8 min) (Lantern) WILLIAM TRAGER AND R. BARCLAY MCGHEE, Rockefeller Institute, Princeton.

44. Infection of the Immature White Mouse with the Avian Parasite, *Plas-*

modium lophurae. (7 min) (Lantern) (Also by Demonstration) R. BARCLAY MCGHEE (Introduced by William Trager).

45. The Mouth Parts of *Liponyssus bacoti* Hirst. (15 min) (Lantern) (Also by Demonstration) FLORA E. GORIROSSI, The University of Texas, Medical Branch, Galveston.

50. The Incidence of Blood Parasites in Liberia. (10 min) (Lantern) MARTIN D. Young, National Institutes of Health, Columbia, S. C.

51. The Parasitemia in Experimental Toxoplasmosis. (15 min) (Lantern) LEON JACOBS AND FRANCES E. JONES, National Institutes of Health.

52. Exoerythrocytic Forms in Relation to Paludrine Administration in Pigeons Infected with *Plasmodium relictum.* (15 min) (Lantern) W. B. REDMOND AND E. L. FINCHER, Emory University.

53. The Antimalarial Activity of Aureomycin against *Plasmodium gallinaceum* in the Chick. (12 min) (Lantern) G. ROBERT COATNEY, JOSEPH GREENBERG, W. CLARK COOPER AND HELEN LOUISE TREMBLEY, National Institutes of Health.

54. Respiratory Organs of Chiggers. (10 min) (Lantern) G. W. WHARTON, Duke University.

55. Chronic Studies on the Pyrethrum Synergist, Piperonyl Butoxide, and their Bearing on Use of These Two Insecticides in the Control of Arthropod Pests. (15 min) (Lantern) MERRITT P. SARLES, U. S. Industrial Chemicals, Inc., Baltimore.

THURSDAY AFTERNOON SESSION, DECEMBER 29, 2:00 PM, COLUMBIA UNIVERSITY, SCHERMERHORN HALL, MORNINGSIDE HEIGHTS, Room 501.

Read

56. The Behavior of the Adult Female Snake Mite, *Ophionyssus natricis,* in Response to Directed Light and the Effects of Body Weight on these Reactions. (15 min) (Lantern) JOSEPH H. CAMIN, The Ohio State University.

57. Temperature, Oribatid Mites and the Development of *Monoecocestus* (Cestoda: Anoplocephalidae), (10 min) (Lantern) REINO S. FREEMAN, University of Minnesota.

58. Preliminary Studies on the Intermediary Metabolism of the Cestode *Hymenolepis diminuta.* (15 min) (Lantern) CLARK P. READ, The Rice Institute.

59. Observations on the Nervous System of the Cestodes. (10 min) (Lantern) NATHAN W. RISER, University of Pennsylvania.

60. Interrelations of *Diphyllobothrium* with Fish-Eating Birds of Northern Lake Michigan. (15 min) (Lantern) LYELL J. THOMAS, University of Illinois.

61. Infections of Chickens with Cysticercoids and Infected Intermediate Hosts of *Raillietina cesticillus.* (5 min) (Lantern) KATHEL B. KERR, Dr. Salsbury's Laboratories, Charles City, Iowa.

62. A Plate Method of Screening Chemicals as Molluscicides. (5 min) (Lantern) DONALD B. MCMULLEN, 406th Med. Gen. Lab. and University of Oklahoma Medical School.

63. Seasonal Studies on *Schistosoma japonicum* in the Intermediate Host, *Oncomelania nosophora.* (10 min) (Lantern) DONALD B. MCMULLEN, 406th Med. Gen. Lab. and University of Oklahoma Medical School; T. ENDO-ITABASHI,

406th Med. Gen. Lab.; S. Seto, 406th Med. Gen. Lab.; S. Komiyama, Yamanashi Health Department, Kofu, Japan; Paul R. Stone, 406th Med. Gen. Lab.

65. Protection Experiments with Copper Oleate Ointment against Schistosomiasis. (7 min) (Lantern) E. Kaufman, G. W. Hunter, III, and C. Pan, 406th Medical General Laboratory.

66. Multiplication of Germinal Cells in the Rediae of *Clinostomum marginatum.* (15 min) (Lantern) W. W. Cort, The Johns Hopkins University; D. J. Ameel, Kansas State College; and Anne Van der Woude, University of Michigan.

67. Parasites of Northwest Wisconsin Fishes. (8 min) Jacob H. Fischthal, Triple Cities College of Syracuse University.

68. Clonorchiasis: Report of a Case in a Caucasian Patient Observed in Boston. (10 min) Donald L. Augustine, Harvard School of Public Health, and Howard J. Isenberg, Beth Israel Hospital, Boston.

69. The Relationship of Male Worms to the Sexual Development of Female *Schistosoma mansoni.* (10 min) (Lantern) Donald V. Moore, Tamarath K. Yolles, and Henry E. Meleney, New York University College of Medicine.

70. The Fate of Dermatitis-Producing Schistome Cercariae in Laboratory Animals. (15 min) (Lantern) Louis J. Olivier, National Institutes of Health.

71. Partial Development of *Echinorhynchus coregoni* in *Hyalella azteca* and the Cellular Reaction of the Amphipod to the Parasite. (10 min) (Lantern) D. L. De Giusti, Wayne University.

72. A Gregarine Parasitic in the Amphipod, *Hyalella azteca.* (5 min) (Lantern) Peter J. Batten and D. L. DeGiusti, Wayne University.

By Title

32. Biological Studies on *Schistosoma mansoni.* José F. Maldonado, School of Tropical Medicine, San Juan.

64. Immunologic Studies, I. Experiments with Bird and Human Schistosomes. G. W. Hunter, III, L. S. Ritchie, W. D. Tigertt, S. Lin, C. Pan, and H. Tanabe, 406th Medical General Laboratory, Tokyo, Japan.

73. Failure to Demonstrate Precipitins in Dogs Infected with *Endamoeba histolytica.* J. C. Swartzwelder and G. R. Muller, Louisiana State University School of Medicine.

74. Cerebral Hydatid Infection. Report of a Case which Recovered Following Surgical Removal of the Parasite. G. C. Anderson (Deceased) and J. C. Swartzwelder, Louisiana State University School of Medicine.

75. Culture Experiments on Intestinal Flagellates. V. Some Additional Longevity Records to September 1, 1949. D. H. Wenrich, University of Pennsylvania.

76. Studies on Encystation of *Endamoeba histolytica.* I. Size of Inoculum, Rate of Multiplication and Density of Population Compared to Degree of Encystation. Martha Grace Everritt, The Tulane University of Louisiana.

77. Studies on Encystation of *Endamoeba histolytica.* II. Influence of Density of Population vs. Rate of Multiplication on Encystation. Martha Grace Everritt, The Tulane University of Louisiana.

78. Action of Neomycin on Protozoa. Sachiko J. Ishihara and Oscar Felsenfeld, Hektoen Institute, Chicago.

79. An Unusual Strain of *Dientamoeba fragilis*. Viola Mae Young, Hektoen Institute, Chicago.

80. The Effect of Penicillin on the P Strain of *Entamoeba histolytica* and Associated Bacterial Flora, in vitro. Irving Pierce Delappe, Michigan State College, East Lansing.

81. *Entamoeba histolytica* Infections in Young Chicks. M. J. Miller, Macdonald College, Quebec.

82. *Giardia* Infection in a Chinchilla. Banner Bill Morgan, University of Wisconsin.

83. The Relationship between Numbers of Adult *Trichinella spiralis* in the Small Intestine and the Precipitin Titer of Mice Given Various Test Infections. James R. Hendricks, University of North Carolina.

84. Comparing in Mice the Percentage Development of *Trichinella spiralis* Larvae Obtained from a Recent and from an Old Infection in Rats. James R. Hendricks, University of North Carolina.

85. Tests in Mice to Determine the Relationship of Intestinal Emptying Time and Natural Resistance to Infection with Pig Ascaris. John E. Larsh, Jr., University of North Carolina.

86. Studies on the Life History of *Capillaria annulata* (Molin, 1858) Cram, 1926. Rex W. Allen, U. S. Bureau of Animal Industry.

87. *Necator americanus* and *Ancylostoma duodenale* Infections in Puerto Rico. José Oliver-Gonzalez, José M. Dobal, and Carlos J. Thillet, School of Tropical Medicine, San Juan.

88. Diet of Hens and Development of *Ascaridia galli* in their Chicks. A. C. Todd and M. F. Hansen, Kentucky Agricultural Experiment Station.

89. The Effect of Inhibitions, Intermediates and a Stimulant upon the Oxygen Consumption in *Ascaridia lineata*. J. Cristopher Mitchell, S. Milton Nabrit, Atlanta University, and B. F. Smith, Spelman College, Atlanta.

90. An Iron Alum-Picric Acid-Hematoxylin Stain for Parasites in Tissues. Morris Goldman, Communicable Disease Center, Atlanta.

91. The Effect of Pregnancy on the Natural Resistance of Mice to *Hymenolepis* Infection. John E. Larsh, Jr., University of North Carolina.

92. *Cysticercus fasciolaris* in the Wild Rat. Kenneth E. Dye, Harold M. Kemple and Wayne W. Wantland, Illinois Wesleyan University.

93. Antibiotics as Bacteriostatic Agents for the Cultivation of Cestodes in vitro. W. Malcolm Reid and Janet I. Boles, Marine Biological Laboratory, Woods Hole, and Monmouth College, Monmouth, Illinois.

94. Experimental Infections with Cysticerci of *Taenia taeniaeformis* in Laboratory Animals. Everett L. Schiller, University of Wisconsin.

95. Gross Parasitism in a Young Racoon. Everett L. Schiller and Banner Bill Morgan, University of Wisconsin.

96. Fluctuation in the Glycogen Content of the Cestode, *Hymenolepis diminuta*. Clark P. Read, The Rice Institute.

97. Experimental Tapeworm (*Moniezia expansa*) Infections in Young Lambs. K. C. Kates and A. Goldberg, U. S. Bureau of Animal Industry.

98. *Quickella* (Family Succineidae), a New Host for Sporocysts of *Leuco-*

chloridium (Trematoda: Brachylaemidae) in Southeastern Michigan. Irving G. Kagan, University of Michigan.

99. The Presence of *Metorchis conjunctus* in Maine. Marvin C. Meyer, University of Maine.

100. Parasites of the Ranidae (Amphibia). XVIII. A. C. Walton, Knox College.

101. Parasites of the Ranidae (Amphibia). XIX. A. C. Walton, Knox College

102. Parasites of the Ranidae (Amphibia). XX. A. C. Walton, Knox College.

103. The Incidence of Parasites of *Rattus norvegicus* in Wisconsin. Everett L. Schiller and Banner Bill Morgan, University of Wisconsin.

104. A Check List of Parasites of Horses in Kentucky. L. S. Olsen, A. C. Todd and M. F. Hansen, Kentucky Agricultural Experiment Station.

105. Parasitological Surveys in the Far East. VI. An Epidemiological Survey of Kyushu Island, Japan. L. S. Ritchie, G. W. Hunter, III, C. Pan, M. Yokogawa and J. T. Szewczak. With the Technical Assistance of S. Asakura, M. Shimizu and F. Verre, 406th Medical General Laboratory, Tokyo, Japan.

106. Parasitological Studies in the Far East. VII. An Epidemiological Survey in Southern Korea. G. W. Hunter, III, L. S. Ritchie, I. C. Chang, W. D. Rolph, Jr., H. C. Mason and J. T. Szewczak. With the Technical Assistance of W. H. Lee, H. Y. Lee, K. S. Cho, S. T. Kim, H. S. Lee, Y. S. Cho, H. F. Chung, Y. H. Han, O. Y. Kim, and O. H. Kim, 406th Medical General Laboratory, Tokyo, Japan.

107. Gregarines Parasitic in Bermuda Marine Crustaceans. Gordon H. Ball, University of California at Los Angeles.

108. Species of *Nematopsis* in *Ostrea virginica*. Victor Sprague, Texas A. and M. Research Foundation and Young Harris College, Young Harris, Georgia.

AUTHOR INDEX

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ABSTRACTS

1. *Phosphorus Deficiency a Limiting Factor in Fowl Parasitism.* J. E. Ackert and S. M. Gaafar, Kansas State College Manhattan, Kansas.

Studies on the effects of mineral supplements on the resistance of chickens to the nematode, *Ascaridia galli,* give evidence that a deficiency in phosphorus not only affects the chickens, but also the ascarid parasites.

Rations high in calcium content and low in phosphorus given to chickens parasitized with approximately 200 *A. galli* eggs resulted in average ascarid infections of 3.3, 2.5, 10.5 and 3.3 worms, respectively, in four consecutive experiments. Whereas, control chickens on optimal amounts of calcium and phosphorus yielded worm averages as follows: 9.6, 7.1, 24.2 and 6.4.

To determine whether the reduced number of *A. galli* was due to the high calcium or the low phosphorus, a group of control chickens was injected with calcium boro-gluconate intramuscularly throughout the period of the experiments with the result of an average of 8.1 worms as compared with 3.3 in the high calcium-low phosphorus group and with 6.4 worms in the controls.

The results of these four experiments on 225 young chickens indicate that a deficiency of phosphorus in the ration is a limiting factor in ascarid infections of fowls.

2. *Lethal Effects of Acetic Acid on Larvae of* Ancylostoma caninum *in Fecal-Soil Cultures.* J. E. Ackert and F. L. Ligenzowski, Kansas State College, Manhattan, Kansas.

Results of 18 tests indicate that a high percentage of larvae of *Ancylostoma caninum* in fecal-soil cultures may be killed by the application of acetic acid at concentrations ranging from 10 to 2.5 per cent.

We used cats as the hookworm hosts and open moist chambers as the fecal-soil culture dishes, and control cultures of known fecal weights for periods ranging from 5 to 8 days. The larvae were isolated from the cultures with the aid of the Baermann apparatus.

The application of 10 per cent acetic acid to known weights of the feces in the cultures yielded an average of less than one larva per gram of feces. The use of 5 per cent of acetic acid resulted in the isolation of approximately 5 larvae per gram of feces, while a dilution of 2.5 per cent yielded an average of 41 larvae per gram. The control cultures yielded an average of approximately 1500 larvae per gram.

That the acetic acid acts on the larvae was shown by results from two preliminary tests. Of about 100 larvae introduced into each culture, 2 each were recovered from the acetic acid cultures and 86 and 82, respectively, from the control cultures.

3. *A New Species of the Free-Living Nematode Genus* Rhabditis *of Interest in Comparative Physiology and Genetics.* Ellsworth C. Dougherty,[1] University of California, Berkeley, and California Institute of Technology, Pasadena; and Victor Nigon, Faculté des Sciences P. C. B., Paris.

Rhabditis briggsae sp. nov. *Synonymy.—Rhabditis elegans,* of Dougherty and Calhoun, 1948 (Proc. Helminth. Soc. Wash. **15:** 55–68); *Rhabditis elegans brevis* Nigon, 1949 (*nomen nudum*) (Comp. Rend. Acad. Sci. **228:** 1161–1162). *Diagnosis.—Rhabditinae:* predominately hermaphroditic, agile worms (with rare males); *Males:* occurring (at temperature of 22° C or less) about 1 per 1400 hermaphrodites; bursa peloderan and cardioid in ventral view with 8–9 pairs of papillae arranged, on each side, in preanal group of 2, slightly postanal group of 3 or 4 (of which, in the former case, the first may sometimes be seen to have a double termination), and terminal group of 3 (including phasmids); measurement (after 8 days at 16° C)—length 0.95–1.2 mm., maximum width 55μ, length of spicules 32–38μ; XO sex-chromosome type. *Hermaphrodites:* usually self-fertilizing; maximum number of eggs in uteri, 12; measurements—length 1.15–1.53 mm., maximum width 90μ, average position of vulva 52% from anterior end; chromosome number, 2n = 12. *Type locality.*—Stanford University campus, Stanford, California. *Discussion.*—Species named for discoverer, Miss Margaret P. Briggs. Isolated from peanut butter enrichment of soil. Very close to *R. elegans* Maupas, 1900, but consistently different in minor respects: male *R. elegans* has 3rd pair of bursal papillae isolated from 4th–6th, and hermaphrodite characteristically twice as many eggs in uteri. Both species differ from others of *Rhabditis* in cardioid outline of bursa. Recently, *R. briggsae* has been grown indefinitely on an axenic medium (sterile pieces of chick embryo), and the first morphological mutant in the Nematoda isolated.

[1] While in France: Fellow of the John Simon Guggenheim Memorial Foundation, 1947–49, and U. S. National Cancer Institute Postdoctorate Fellow, 1947–48. At present: Senior Research Fellow of the American Cancer Society as recommended by the Committee on Growth of the National Research Council, 1949–52.

4. *Some Abnormal Host Relationships of* Nippostrongylus muris. William D. Lindquist, Michigan State College, East Lansing, Michigan.

In infections with *Nippostrongylus muris* in the cotton rat, golden hamster, and guinea pig a very small percentage of stunted adults developed in the first two hosts while in the third the worms migrated through the lungs but none grew to maturity. Sections of the skin and lungs of the first two initially infected abnormal hosts showed an intense cellular reaction which caused the retention and encapsulation of larvae. In a small series of slides of the hamster the cellular reaction and retention was demonstratd only in the skin. As early as 19 hours after infection larvae in the cotton rat were found surrounded by wandering and polymorphonuclear cells and later they became completly encircled by fibrous tissue. This cellular reaction resulting in encapsulation and destruction of larvae in the skin and lungs appears to be one of the mechanisms by which nematodes that penetrate the skin are prevented from developing to maturity in abnormal hosts. It shows a striking similarity to the reaction that occurs in the skin and lungs of a normal host, the laboratory rat, which has been repeatedly infected with large numbers of larvae of *N. muris*. In this case the cellular reaction and encapsulation of larvae is supposed to be in some way related to the presence of specific antibodies in the immunized animal. It is suggested that the encapsulation of larvae in initial infections in the abnormal hosts is not related to the presence of antibodies but may be due to the slowing down of their physiological processes in an unfavorable environment which permits them to be caught by a foreign body reaction.

5. *The Results of Feeding Small Amounts of Phenothiazine in Pure Infections of the Nodular Worm* (Oesophagostomum radiatum) *in the Calf.* Roy L. Mayhew, Louisiana State University.

The calves used in these experiments were raised parasite free and inoculated with pure cultures of infective nodular-worm larvae. From 10 to 20 fecal examinations (usually daily) were made after eggs began to appear in the manure before beginning the experiment. One and one-half grams of phenothiazine was mixed with the grain concentrate at the regular evening feeding for 6 days to 4 calves and for 14 days to three others.

Daily fecal examinations failed to demonstrate the presence of eggs in the manure in all animals except on (No. 194) in from 6 to 14 days after the first feeding of the drug. In the case of No. 194, one of the animals fed for 6 days, the number of eggs became greatly reduced after 6 days, and 4 days after the beginning of a second 6 day feeding 21 days later they disappeared altogether.

Abnormal eggs (irregular cell divisions, failure to divide, dark pigmentation, and degeneration) were noted in manure samples from one group of animals taken 16 hours after the first feeding. Abnormal eggs were not noted in samples taken from another group of animals approximately 8 to 10 hours after the first feeding, but were detected in samples collected 18 to 20 hours later.

One calf that was fed 6 days and kept 8 weeks under controlled conditions still remained negative. Another animal remained negative 7 weeks, after which time a relatively small number of eggs was recovered from the manure. Two animals that had been fed 6 days were killed after 10 negative daily fecal examinations were obtained and adult nodular worms were removed from both animals.

6. *Thyroid Abnormalities as a Possible Factor in Trichostrongylidosis.* John H. Whitlock, New York State Veterinary College, Ithaca, New York.

During the past three year's experiments with natural infections of trichostrongyles involving over 220 sheep, six classical cases of trichostrongylidosis have occurred. These were characterized by anemia, market emaciation and, in some cases, intermittent diarrhoea. In the 1947 experiments both ewes and lambs were fed ordinary salt, and three cases occurred. Two of these were examined by routine post mortem technic and showed the expected lesions although the immediate cause of death in one lamb was an intussusception initiated by an abnormally long Meckel's diverticulum. The third case, twin to the last named lamb, was examined with extreme care. The thyroids were markedly enlarged and sections showed a typical colloidal goitre. In 1948 the lambs were fed a balanced mineral mixture including iodine but the ewes while pregnant were fed ordinary salt. Two cases of typical trichostrongylidosis occurred both showing an embryonal type of thyroid. In 1949 ewes and lambs were generally kept on iodized salt with the exception of one ewe which received only common salt during pregnancy. The twin lambs from this ewe were put on a course of thiouracil, one was kept relatively free of parasites by anthelmintics, the other was allowed to develop a natural infection. The latter lamb died and constitutes the only case of marked early trichostrongylidosis in the flock for this year. In addition to markedly embryonal colloid-free thyroids this lamb had a patent foramen ovale.

These data strongly suggest that an important primary cause of trichostrongyldosis is thyroid deficiency, and that the prenatal form is the more serious.

7. *Preliminary Observations on the Life History of* Ascaris columnaris. JACK D. TINER, University of Illinois.

Eggs of *Ascaris columnaris* taken from a racoon *Procyon lotor* near Urbana, Illinois, embryonated, and administered to rodents consistently produced the "Gehirnsymptome" reported by Fülleborn (Arch. Schiffs. u. Tropenhyg. 25: 62–63). Fatally terminating central nervous system disturbances first became evident 9 to 25 days after infective eggs were fed to grey squirrels *Sciurus carolinensis,* white footed mice *Peromyscus leucopus,* house mice *Mus musculus,* cotton rats *Sigmodon hispidus,* hamsters, and quinea pigs. Larval ascarids were demonstrated in press preparations of the brains of a series of mice, and injections of various tissue suspensions from diseased to healthy rodents failed to demonstrate any other transmissible agent. Four of the six grey squirrels showed ante-mortem symptoms of nervous system impairment. Encapsulated larval ascarids were found in the heart muscle, pericardium, walls of the caval veins, and to a lesser degree in the lungs and under the pleura of the two surviving squirrels. Two fox squirrels *Sciurus niger rufiventer* from the woodlot where the racoon was taken had similarly located larval ascarids in their thoracic viscera.

Smaller numbers of rodent hosts were given infective eggs of *Ascaris lumbricoides* (porcine origin), *Toxocara mystax,* and *Parascaris equorum.* No symptoms of nervous disorder were observed during the twenty-five to thirty days that the animals were watched following experimental feedings.

Cotton rats containing 15 to 20 day-old cysts of *A. columnaris* were fed to ascarid-free racoons and skunks and infections were established in the final hosts. Three *P. leucopus* containing cysts about 25 days old were fed to a skunk *Mephitis* sp. from Cheboygan Co., Michigan. The animal died on the forty-fourth day with 485 young, but mature adult ascarids in its intestine.

8. *Observations on the Pathogenicity of* Nematodirus spathiger *in Lambs.* K. C. KATES AND J. H. TURNER, U. S. Bureau of Animal Industry.

Various clinical manifestations in sheep have been ascribed to heavy natural infections with *Nematodirus* spp. The one reported attempt to confirm these observations experimentally was unsuccessful (Kauzal, 1937). Recently, several cases of heavy natural infections with *N. spathiger* have appeared in lambs at Beltsville, Md. Clinical symptoms usually consisted of diarrhea, poor weight gains, weakness, emaciation, and occasional deaths. *Trichostongylus* spp., usually associated with verminous diarrhea in lambs, were not present, but light infections of other helminths and occasional heavy infections of coccidia were observed. One lamb, which had never shown diarrhea, died suddenly. It harbored over 30,000 *N. spathiger* and insignificant numbers of other parasites.

Preliminary experimental infections were accomplished by a single feeding of 12,000 to 105,000 *N. spathiger* larvae to 9 parasite-free lambs, without observable ill effects. In a controlled experiment 5 lambs were fed 300,000 to 900,000 larvae each, over a 3-day period; uninfected controls were maintained under identical conditions. Infected lambs developed diarrhea 11 to 14 days after infection, went off feed, became weak, emaciated, and made poorer weight gains than the controls. Duration of diarrhea was 7 to 14 days, terminating before the 29th day after infection; anemia was not observed, and no deaths occurred. Although heavy infections were established, as determined by fecal egg-counts, few worms were recovered at autopsy 13 weeks after infection, indicating a rapid loss of the original infection. At autopsy, the total weight of the infected lambs was still 44.5 pounds less than that of the controls.

9. *A Nephelometric Method of Calibrating the Photo-Electric Light Meter for Making Egg-Counts by Direct Fecal Smear.* PAUL C. BEAVER, Tulane University.

A suspension of $BaSO_4$, one drop (0.05 cc) of which has the same turbidity on a microscope slide as the standard fecal smear described by Beaver (1949, J. Parasit. 35: 125–135) as containing 1/300 cc of formed stool can be produced as follows: To 2 parts N/1 $BaCl_2$ solution add 1 part pure glycerine (glycerol) and to 2 parts 2N Na_2SO_4 solution add 1 part pure glycerine; then at room temperature add drop by drop with constant stirring 2 parts of the $BaCl_2$-glycerine mixture to 3 parts of the Na_2SO_4-glycerine mixture and allow to stand with occasional shaking for 24 hours or more. The suspension remains constant for many weeks and can be exactly duplicated. Thus, diverse types of photo-electric light meters may be calibrated for making egg counts by direct smear, and by using the same $BaSO_4$ suspension the accuracy of the instrument can be checked from time to time.

10. *Effect of purified diets on the host-parasite relationship of chickens to* Ascaridia galli. ELVIO H. SADUN, JOHN R. TOTTER AND CECILIA K. KEITH, University of Arkansas.

A total of 109 chickens was used to study the effect of vitamin deficiency on the host's resistance. Of these, 57 were reared on a highly purified diet deficient in pteroylglutamic acid (PGA), 25 on the same diet to which an adequate amount of PGA had been added and 27 on a commercial crude diet. Three weeks after inoculation with *A. galli* the deficient chickens harbored significantly more and longer worms than those which received an adequate amount of PGA, thus indicating that a PGA-deficiency brings about a lowered natural resistance to this parasite.

The effect of purified diets on the growth of worms was also studied. Chickens reared on a purified diet adequate for good growth, three weeks after inoculation harbored worms that were 2.7 mm. long while those on a crude diet harbored worms that were 21.4 mm. long. Since the purified diet used in these experiments contained minimal amounts of Vitamin B_{12}, further studies were carried out with the addition of 2 per cent whole dried liver in one group, and 8 ml. of 15 unit liver extract per kilo of diet in another. At necropsy, three weeks later, the lengths were as follows: purified diet plus dried liver, 3.4 mm.; purified diet plus liver extract, 11.1 mm.; commercial crude diet, 27.7 mm. These results indicate that there is a substance present in the liver extract necessary in relatively large amounts for the normal growth of the worms. This substance may be Vitamin B_{12} or the animal protein factor.

11. *The Cultivation of the Free-Living Stages of Parasitic Nematodes in the Absence of Living Bacteria.* Paul P. Weinstein, Johns Hopkins School of Hygiene. (Now with National Institutes of Health).

Ancylostoma caninum, A. duodenale, and *Nippostrongylus muris* were cultured from the egg to the filariform stage in the absence of living bacteria using either fresh chick embryo or rat liver extracts containing penicillin and streptomycin. *A. caninum* larvae obtained from rat liver extract cultures gave rise to a normal infection in a puppy. Small increases in the concentration of tissue extracts at the lower concentration level (5 per cent) caused comparatively greater increases in growth than did large increases in the higher concentration range (25 per cent). Extracts passed through a Seitz filter completely lost their ability to stimulate growth. Heating rat liver extract to either 55° C. for 15 minutes or to 100° C. for one minute inactivated the growth-promoting substance(s) almost entirely for all three species. Dialysis of embryo extract profoundly altered its ability to support growth and to maintain *A. caninum* larvae. None showed any increase in size and market derangement of lipid metabolism manifested by the accumulation of large fat globules resulted. Autolysis quickly followed. Both *A. caninum* and *A. duodenale* larvae were cultured in the sediment obtained by the centrifugation of rat liver extract at approximately 27,000 x g, which was dissolved in Tyrode's solution. They exhibited development almost identical to that occuring in dialyzed extract. Newly hatched larvae cultured in the supernatant, however, were maintained in good condition for many days, but showed no sign of growth. No such sharp differences were apparent using the two comparable fractions from chick embryo extract. Data were obtained indicating that rat liver extract may at times contain a factor which acts as a powerful growth inhibitor.

12. *Immunity to Reinfection in Trichinosis.* Irving Rappaport, Long Island College of Medicine, and Helen S. Wells, School of Public Health, Columbia University.

In several series of experiments, mice were given an initial infective dose of 100 *T. spiralis* larvae. This was followed by a single reinfective dose of 800 larvae after either 10 days, 35 days or 3.5 months. In one series, 300 larvae were administered 35 days after the original infection. The immunity was studied during the entire course of the intestinal phase by counting the number and measuring the lengths of the adult worms. The muscular phase was studied by dilution counts of larvae after digestion in a mixture of pepsin and dilute hydrochloric acid.

A comparison of reinfected and control mice demonstrated no apparent reduction in adult worm yields in animals reinfected 10 days after initial infection. Worm measurements were not made in this series. With mice reinfected after 35 days or after 3.5 months, the yields of adult worms were somewhat lower than in control mice, but no great initial loss of worms occurred. On the average, male worms were only slightly smaller in reinfected animals. Female worms in reinfected animals showed considerable diminution in size. Results of muscle larval counts indicated that considerably fewer larvae became encysted in reinfected animals than in controls.

The above findings are not in accord with results obtained by other workers who believe that immunity is manifested by an immediate loss from the intestine of a large proportion of the challenging dose.

13. *Effect of Low Temperatures on Acquisition of Parasites by Swine.* John S. Andrews, U. S. Bureau of Animal Industry, Tifton, Georgia.

In studies of the acquisition of parasites by growing pigs, conducted at the Georgia Coastal Plain Experiment Station 1941 to 1946, fall-farrowed pigs were maintained after weaning on a temporary pasture of green oats and fed shelled corn, protein supplements, and minerals. Fecal examinations by the Stoll technique were made at weekly intervals. The pigs were slaughtered at an average weight of 225 pounds and examined to ascertain the number infested with parasites and the number of worms harbored.

Analysis of the findings in relation to air temperatures prevailing during the period between farrowing and slaughter revealed the existence each year of a positive correlation between the incidence and size of infections with certain parasites and the minimum air temperature. The parasites so affected were one species of nodular worm, *Oesophagostomum dentatum*, the whipworm, the kidney worm, the lungworms, and the large intestinal roundworm or ascarid; correlations for the last-named parasite are based on the egg count data. The correlations varied from 0.68 for the incidence of infection with kidney worms to 0.97 for the numbers of ascarid eggs per gram of feces. The findings indicate that (1) 20° F. was the critical temperature at which a reduction in the acquisition of parasites occurred, and (2) a reduction in the number of parasites acquired by the pigs occurred even though the temperature named persisted only a few hours each day. Infective stages of the parasites affected are, in general, located in the top inch of soil and would, therefore, readily be affected by adverse temperatures.

14. *Giant Kidney Worm,* Dioctophyma renale, *in a Dog.* George L. Graham and James H. Mark, School of Veterinary Medicine, University of Pennsylvania.

A year old English bulldog showed symptoms observed initially on February 4, 1949. There was haematuria, marked frequency of urination and convulsive shivering. The dog failed to respond to symptomatic treatment and was hospitalized on February 16th with a tentative diagnosis of hemorrhagic cystitis. The animal was anemic (Hb 8.9 grams), showed a palsy-like convulsion of the head and was obviously suffering considerable pain. Examination of urine sediment revealed *Dioctophyma renale* eggs. Following transfusion, a nephrectomy of the right kidney was performed. It contained a female worm measuring 86 centimeters. The anterior pole of the kidney was perforated; the capsule thickened, outpouched and perforate. Areas on the serosa of the abdominal viscera were covered with granulation tissue. Recovery was rapid and uneventful. All symptoms disappeared within two days and the animal was discharged three days later. The animal's preoperative weight was 42 pounds; when discharged, 55 pounds. Two months earlier the animal had seemed in excellent health and weighed 65 pounds.

The dog had lived in the city all its life except for the month of August, 1948 when it was taken on a trip, the last two weeks of which was spent at a camp on the French River in Ontario, Canada. The animal was very fond of raw fish, ate fish entrails given to him and even dug up and ate entrails of catfish which had been buried. He also drank freely from the river. The history suggests no other possible exposure to sources of infection.

15. *Evidences of Acquired Immunity in the Cotton Rat to Infection with the Filarial Worm,* Litomosoides carinii. Etta Mae Macdonald and J. Allen Scott, University of Texas Medical Branch, Galveston, Texas.

Controlled experiments have shown that all previously uninfected cotton rats so far tested can be heavily infected with this worm. Exact quantitative testing has been possible by dissecting infective larvae from mites in Tyrode's solution, transferring them to a cover glass supported by blocks, where they were carefully examined to remove immature or damaged forms. They were then counted and flushed into a subcutaneous pocket through an incision in the skin of the rat. Maturing worms found at autopsy have accounted for 50 per cent or more of the larvae given in this way. Certain cotton rats were given a series of such infections and after time for the worms to reach maturity, a single test infection was given similarly to each of these rats as well as to each of a series of previously uninfected control rats. Both groups were autopsied together when the worms of the test infection would be about half grown and thus distinguishable from the worms previously introduced. There was no marked difference between the experimental and the control groups in the percentage of worms developing, but there were certain differences in the growth rates of the worms. As compared with those in the control rats, the worms in the previously infected rats were shorter and less mature on the average and a few individuals showed markedly retarded growth. This work was supported by United States Public Health Service Research Grant RG1297 and Office of Naval Research Contract N8onr 61,000.

16. *Intestinal Flagellates of a Wallaroo,* Macropus robustus. Harold Kirby and Bronislaw Honigberg, University of California.

A trichomonad with five anterior flagella, named *Pentatrichomonas macropi,* was reported by Tanabe (1926) and Herman (1939) from kangaroos in zoos in the eastern United States. In

the wallaroos in San Francisco, no five-flagellate trichomonad has been found, but in cultures from faeces a species of *Trichomonas* with four anterior flagella has been grown repeatedly. Except for the absence of the independent anterior flagellum, it has much resemblance to the common intestinal trichomonad of man. The parabasal body in both flagellates is a small, rounded structure applied to one part of a parabasal filament several microns long. In the walleroo flagellate it is about a micron or a little more in longest diameter; in the flagellate of man it may be smaller. There is a well-developed pelta in both flagellates. A species of *Retortamonas* has also been cultured from wallaroo faeces, though it is less likely to develop and persist than is *Trichomonas*. The two flagella are about equal in length and thickness, and are both of the acroneme type, as is the posterior flagellum of many species of trichomonads. A species of *Monocercomonas* has been found in small numbers in certain cultures from the wallaroo faeces. It is distinctly different from *Trichomonas* in pelta and parabasal body, besides having a separated recurrent flagellum and three anterior flagella.

17. *A Contribution to the Discussion about the Relationship of* Chilomastix *and* Retortamonas. Harold Kirby and Bronislaw Honigberg, University of California.

Studies of silver-impregnated specimens of a species of *Retortamonas* from the wallaroo have contributed materially to an understanding of the cytostomal structure. The cytostomal fibril of *Retortamonas* is single, continuous, rounding the anterior edge of the cytostome, with two approximately equal arms along the sides, anteriorly bordering a curved triagular or trapezoidal membrane that covers the anterior part of the cytostomal depression. An essentially similar cytostomal border was reported by us in *Chilomastix magna,* though the comp'exity of the cytostomal structures is greater in that larger flagellate. The structures shown by Nie (1948) in *Chilomastix intestinalis* and other species appear comparable, though his interpretation differed somewhat. The relationship between *Retortamonas* and *Chilomastix* has been discussed by several protozoologists (Mackinnon, Alexeieff, Wenrich), beginning a good many years ago. Alexeieff (1917) and Wenrich (1931, 1932) proposed placing the two genera in the same family. Aside from the presence of two additional anterior flagella in *Chilomastix,* the essential similarity in structural characteristics strongly suggests that relationship. Nevertheless the usual criteria for separation of orders in the non-pigmented Flagellata have generally continued to be followed; then *Retortamonas* goes into one order and *Chilomastix* into another. The relationship between *Retortamonas* and *Chilomastix* is a good illustration of the artificiality of designating the orders Protomonadida and Polymastigida solely on the basis of the number of flagella.

18. *Some New Chemotherapeutic Agents in Experimental Enterohepatitis* (Blackhead) *of Turkeys.* E. Waletzky, M. C. Brandt, A. Bliznick and C. O. Hughes, Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company.

Severe experimentally induced enterohepatitis suitable for the evaluation of potential chemotherapeutic agents has been produced in some 40 experiments with several thousand turkeys by a single rectal inoculation of homogenized infected liver suspension. Only livers showing typical, but non-caseated, "blackhead" lesions were used. They were obtained from large turkeys sacrificed about 14 days after an oral inoculation with embryonated *Heterakis* eggs (from worms in slaughtered chickens), or in some cases 10 days after a rectal inoculation with infected liver material. Such livers were diluted with an equal weight of 0.9% saline, homogenized in a blendor for 30 seconds, chilled, and 1 cc. of the suspension per 100 grams of body weight was inoculated rectally into recipients 2 to 4 weeks of age. About 80% of the latter died with severe cecal and liver lesions after a mean survival time of 11 days in untreated controls.

Many types of drugs with antimalarial (*P. gallinaceum*) or anticoccidial (*Eimeria tenella*) activity were inactive in turkey enterohepatitis, when tested by the drug-diet method with continuous administration beginning before or shortly after inoculation. However, two related compounds (trademarks ENHEPTIN–T and ENHEPTIN–P) were highly active even when treatment was only begun 4 or more days after inoculation. The nature of the compounds and detailed results and their application to the treatment and control of enterohepatitis will be discussed.

19. *The Development of Resistance to and the Effect of Some New Chemotherapeutic Agents on Enterophepatitis Induced by the Oral Administration of Cecal Worm Ova to Chickens and Turkeys.* Sterling Brackett and Alexander Bliznick, Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company.

Chicken flocks in Connecticut and vicinity have been found to be almost universally infested with the cecal worm, *Heterakis gallinae.* Embryonated ova of cecal worms from 20 to 22 lots of chicken ceca, collected at random from poultry markets dealing in local fowl, produced fatal

infections with enterohepatitis in young turkeys. These infections were regularly produced in turkeys inoculated with 250 embryonated ova. Smaller inocula were usually non-infectious, although one bird died with typical liver and cecal lesions, after receiving an estimated 30 ova. The mean survival time was 17 days in unmedicated infections. The drugs ENHEPTIN–T and ENHEPTIN–P (trademarks) (Waletzky, et al., 1949) are as effective in enterohepatitis produced in this manner as in infections produced by the rectal inoculation of infected liver emulsion. Experimental evidence of the development of immunity from enterohepatitis has been obtained in turkeys and will be discussed. The pathological condition of the ceca of turkeys suffering from enterohepatitis is evidently unfavorable for the normal development of *Heterakis gallinae*. This worm, however, does develop well in turkeys "immune" to enterohepatitis.

20. *Observations of* Endamoeba histolytica *in Microcultures of* Trichomonas foetus. Charles W. Rees, Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Maryland.

The recent success of Phillips in cultivating *Endamoeba histolytica* without bacteria in the presence of *Trypanosoma cruzi* has reopened the problem concerning the possibility of growing the amoebae with other species of protozoan organisms. A series of experiments was, therefore, conducted with the objective of cultivating *E. histolytica* with *Trichomonas foetus,* using the petrolatum-sealed microtechnique of Phillips and Rees. Although on several occasions overnight division of a single isolated amoeba has been noted there was no growth beyond that period. Further growth of amoebae may have been inhibited by rapid growth of *T. foetus* which completely predominated in the medium from the second through the eighth days of incubation. Attempts to grow the amoebae with heat-treated *T. foetus* were likewise unsuccessful. Amoebae failed to divide in *T. foetus* cultures that were previously exposed at 46° C. and at higher temperatures, until all of the flagellates were killed. The controls, heat-treated *T. foetus,* with live *Trypanosoma cruzi,* gave good growth of amoebae.

21. *A Highly Efficient Medium for the Cultivation of* Endamoeba histolytica, *Suitable for Drug Testing.* Ernest Hartman, Stritch School of Medicine, Loyola University, Chicago, Ill.

On an *a priori* assumption that not all of the factors and conditions present in a host are necessary for the growth of a parasite and that some host factors may be detrimental to the parasite, studies were undertaken to devise a minimal medium giving good growth of *E. histolytica.*

Using commercial dried egg yolk, yeast extract, and NaCl it has been possible to secure a nearly clear fluid medium which with the addition of dry rice starch will produce population densities of over 80,000 amoebae per ml. Best results have been secured by taking 10 grams of the dried egg yolk, suspending this in 200 ml. of 0.8% NaCl, boiling to coagulate the egg yolk, filtering through muslin (discard the filtrate), suspend the coagulum in 300 ml. of 5% NaCl, place in refrigerator over night, boil, and filter through muslin and then through paper. This constitutes a stock solution which is diluted to a concentration of 0.8% NaCl as needed. Biological assays are made by taking 100, 50, 30, 20, and 10 parts of the diluted stock solution and making up to 100 parts with 0.8% NaCl. Three tenths gram of yeast extract is added to each lot and the pH is adjusted with NaOH to 7.2–7.4. The medium is then tubed and autoclaved. The lot showing the best growth is taken as having the proper dilution of egg yolk extract. The extract produces poor or no growth if it is too concentrated.

It seems that phospho-lipids are necessary constituents in optimum concentration of about 10–20 mg. per cent.

Criteria for judging the suitability of a medium are discussed.

22. *Some Observations on Vitamins and Antibiotics in the Cultivation of* Endamoeba histolytica. E. Clifford Nelson, Medical College of Virginia, Richmond, Virginia.

In a series of trials to eliminate the bacteria from egg-yolk-extract medium cultures of *Endamoeba histolytica* it was found that bacterial growth could be stopped with streptomycin but the amebae also failed to live. The possibility of the lack of some essential growth substance was explored by test of growth after addition of vitamins considered deficient in the medium. A notable response to addition of ascorbic acid and calcium panthothenate was observed in antibiotic free medium. On the addition of streptomycin to ascorbic acid containing medium both bacterial and amebic growth stopped. On the addition of streptomycin to medium containing both ascorbic acid and calcium panthothenate, the antibiotic appeared to be completely inactivated and both bacteria and amebae flourished.

23. *A Stain for the Rapid Differentiation of the Trophozoites of the Intestinal Amoebae*

in Fresh, Wet Preparations. Clarence A. Velat, Paul P. Weinstein, and Gilbert F. Otto, The Johns Hopkins University, Baltimore, Md.

The stain in question is an aqueous solution of the reaction product between crystal violet and haematoxylin. The crystals which form the basis for the stain are obtained by the reaction of hot 1% haematoxylin with warm 2.5% crystal violet in the presence of triethanolamine. The resulting precipitate is, after drying, stable indefinitely. For use it is dissolved and allowed to ripen in an acetate buffer over a 2 to 3 week period and filtered before use. Staining is almost instantaneous at pH 4.2–4.4 but slightly slower as the pH increases. At pH 4.6–4.8 there is critical staining of the nuclei of trophic amoebae in 3 to 5 minutes and at pH 5.2–5.4 in 5 to 15 minutes. In the latter pH range they remain unchanged for at least 72 hours on vaseline sealed preparations.

24. *Observations on the Occurrence of* Rickettsia tsutsugamushi *in Rats and Mites in the Malayan Jungle.* Robert Traub, Lyman P. Frick and Fred H. Diercks, Army Medical Department Research and Graduate School, Washington, D. C.

The United States Army Scrub Typhus Unit in Malaya has isolated *Rickettsia tsutsugamushi,* the causative organism of scrub typhus, from tissues of animals and mites taken in the primary jungle. Isolations were made from the spleens of *Rattus mulleri* Jentink, *Rattus edwardsi* (Thomas), a species of the *Rattus rajah* group and from the trombiculid mite *Euschongastia indica* Hirst collected from the squirrel *Callosciurus nigrovittatus* (Horsfield). This is the first time that mites of the genus *Euschongastia* have been found infected in nature with the rickettsiae of scrub typhus. Similarly, it is the first reported instance of natural infection in these species of rats.

Despite the classical picture of outbreaks in areas of secondary growth, scrub typhus is apparently endemic in dense jungle in Malaya, and a number of species of small mammals and mites are probably involved.

25. *The Oxygen Requirements of Parasites.* James W. Moulder, University of Chicago.

Respiration may be defined as the sum total of the chemical reactions carried out by the living cell which result in the liberation of energy. Of these energy-yielding reactions, by far the most important are those of oxidation-reduction. The energy obtained from a given amount of substrate under anaerobic conditions is usually much less than in the presence of oxygen, and anaerobic metabolism is characterized by a rapid utilization of oxidizable substrates and an accumulation of partially oxidized end products. Most animal parasites appear to be facultative anaerobes. In typical aerobic respiration, the hydrogen and electrons of substrates are transferred to molecular oxygen through the intermediation of pyridinoprotein, flavoprotein, and iron-porphyrin-protein catalysts. In anaerobic respiration, the oxidation of one substrate is coupled with the reduction of another substrate. Some form of carbohydrate is the chief energy source for both the aerobic and anaerobic respiration of animal parasites, and a major portion of this energy is probably conserved and transferred in the form of high-energy phosphate bonds. The blood forms of malarial parasites and trypanosomes inhabit a medium rich in oxygen and do not long survive under completely anaerobic conditions, yet typical incompletely oxidized end products of anaerobic metabolism are formed by these organisms. Malarial parasites appear to have a full complement of respiratory enzymes. The aerobic respiratory enzymes of the pathogenic and non-pathogenic trypanosomes are not the same. The pathogenic trypanosomes probably contain no iron-porphyrin respiratory enzymes. The intestinal protozoa live in an environment low in oxygen, and derive most of their energy from anaerobic oxidations. All of the parasitic helminths probably consume oxygen in the presence of sufficiently high oxygen tensions, but in the low oxygen tensions of the animal intestine, worms such as *Ascaris* must depend mainly upon anaerobic reactions as energy sources. Worms which inhabit the blood and tissues of their vertebrate hosts are exposed to much higher oxygen tensions, but the type of respiratory metabolism exhibited by a given helminth is dependent upon the enzymic makeup of the parasite as well as upon the supply of oxygen. Many parasitic worms apparently consume oxygen without the aid of iron-porphyrin respiratory enzymes. A survey of the oxygen requirements of animal parasites reveals that many of these parasites lead essentially anaerobic lives in environments low in oxygen. Blood and tissue parasites usually have an abundant oxygen supply, and, depending upon their enzymic makeup, may or may not obtain most of their energy from aerobic oxidations. Adaptation to parasitic existence, both aerobic and anaerobic, often seems to be accompanied by the loss of aerobic respiratory enzymes, particularly the iron-porphyrin proteins.

26. *Effect Of Drugs on Metabolism and Enzyme Systems of Parasites.* Ernest Bueding,

Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland, O.

Until some time ago discussion of the action of drugs was limited mainly to a consideration of their usefulness in therapeutics. Since basic mechanisms occurring within the cell have begun to be clarified, information can be acquired concerning the manner in which drugs affect and alter metabolic systems of parasites. There is strong presumptive evidence for the involvement of enzyme systems in the action of many chemotherapeutic agents. Examples illustrating the effect of drugs on the metabolism of parasites will be discussed. However, premature interpretation of the chemotherapeutic action of a drug on the basis of its effect on a particular metabolic reaction should be avoided. For instance, if a drug affects an enzyme system only in a concentration which is much higher than that necessary to produce chemotherapeutic activity, the effect observed *in vitro* cannot explain the action *in vivo*. Thus, continued correlation of observations on isolated metabolic systems with those on the parasite in its normal habitat is required to recognize the significance of the action of drugs on enzymes.

Marked differences between the metabolism of the host and that of parasitic organisms have been observed. Therefore opportunities are available to inhibit essential metabolic processes of the parasite without damaging the host. Furthermore, the biochemical characteristics of parasites vary greatly from one species to another even among organisms closely related morphologically. Such differences explain why frequently chemotherapeutic agents possessing high activity against one parasite have no effect on others, and why it is necessary to investigate the metabolic characteristics of a particular organism against which chemotherapy is directed rather than those of related species of greater availability.

As understanding of the comparative biochemistry of parasites is advanced, the fundamental actions of more and more known drugs will be elucidated and possibilities will arise for the rational development of new chemotherapeutic agents.

27. *The Carbohydrate Metabolism of Parasites.* Theodor von Brand, Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Maryland.

The following phases of the problem will be discussed: Endogenous and exogenous sources of carbohydrates, quantitative aspects of carbohydrate turnover, endproducts of carbohydrate metabolism in aerobic and anaerobic fermentations, and intermediate carbohydrate metabolism. Representative examples will be drawn from endoparasitic protozoa, worms, and arthropods.

28. *Protein Metabolism of Parasites.* Quentin M. Geiman and Ralph W. McKee, Department of Tropical Public Health and Department of Biological Chemistry, Harvard School of Public Health and Harvard Medical School, Boston, Massachusetts.

Proteins, those "primary or preeminent" plant and animal substances, are synthesized during growth and in general constitute the structural matrix and functional enzyme systems of living matter. Protein metabolism must be considered as only one of the "flow" processes of cellular metabolism, resulting from the utilization of amino acids, and action of enzymes. The functioning of a highly integrated system of enzymes and genes for each organism leads to the formation of proteins peculiar to the species. Furthermore, the processes involved are influenced by the metabolism of carbohydrate and lipid.

Exploration of protein metabolism in animal parasites is just beginning. Available information will be presented about the protein metabolism in selected parasitic species representing the arthropods, helminths and protozoa. In searching for suitable examples and data about animal parasites, the diversity of structure, modes of life, types of host, parts of host parasitized, degree of pathogenicity and nature of and duration of immune response required an arbitrary choice of studies having something in common. Since blood and its elements play a considerable role in parasitism, studies with certain blood sucking arthropods (*Rhodnius prolixus,* species of *Glossina* and mosquitoes), blood dwelling (blood flukes) or blood sucking helminths (species of *Ancylostoma*) and blood or tissue dwelling protozoa (species of *Plasmodia, Leishmania* and *Trypanosoma*) have been chosen for a comparative discussion.

The comparison reveals extensive leads for study, but no clear cut pattern of protein metabolism among animal parasites can be plotted because available information is too incomplete. The discussion emphasizes, however, the urgent need for elaborating the type of studies reviewed in this symposium in attempts to determine the physiological role of carbohydrate, lipid and protein metabolism to the mode of life, the basis for host-specificity, pathogenicity, immunity and chemotherapy of animal parasites.

29. *Osler and Parasitology.* Thomas W. M. Cameron, Institute of Parasitology, Macdonald College, Quebec.

Presidential Address.

30. *The Effects of X-Ray on* Rhabditis *Species.* Lyell J. Thomas and Henry Quastler, University of Illinois.

Rhabditis sp., a mematode producing scabies in dairy cattle, was X-ray'd with 10,000, 20,000, and 40,000 Roentgen units. The first generation larvae showed accelerated mutation in the production of differences in refractive indices of sections of the intestine containing rhabditin granules. The nematode may be dried for as long as nine months and revived. X-ray effects in the dry state seem to require twice as much to produce the same effect as in the wet state.

31. *Strain Differences in* Plasmodium gallinaceum *Brumpt.* Joseph Greenberg, Helen Louise Trembley and G. Robert Coatney, National Institutes of Health, Bethesda, Maryland.

The 8A strain of *Plasmodium gallinaceum* was established in this laboratory in 1942 and now 3 distinct strains are distinguishable. The BI and the SP strains differentiated themselves spontaneously in the Bethesda laboratory and the third, the M strain, arose from the BI strain in the Memphis laboratory (Haas *et al.,* 1948, J. Parasit. 34: 306–320). The M strain produces few or no phanerozoites when mosquito-passaged. Each of the other strains produces abundant phanerozoites when passaged by mosquitoes or by the inoculation of infected blood. Merozoites arising from phaneroboites of the BI strain are unable to produce normal parasites in the erythrocytes; those which are found are small and unpigmented. Merozoites arising from phanerozoites of the SP strain, on the other hand, are able to invade the erythrocytes and produce a typical parasitemia. These characteristics apply to both the phanerozoites of the blood-passaged and mosquito-passaged infections. These strains have been interconverted by single oocyst inoculation or by serial blood transfer.

32. *Biological Studies on* Schistosoma mansoni, José F. Maldonado, School of Tropical Medicine, San Juan, Puerto Rico.

Following studies on the hatchability of the egg and the longevity and infectivity of the miracidium of *S. mansoni,* two other aspects have been studied. Investigations on the survival of the egg in undiluted stools under diverse environmental conditions have demonstrated that in the laboratory very few eggs remained alive after 4 days, the average life span being 2 to 3 days. In the field, under shade, some survived up to eight days under favorable weather, but in the absence of rain they perished within two days. Direct sunlight killed all the eggs by the second day. In latrine pits containing a certain amount of water the average survival was about five days.

Light has been found to have a decided effect on hatching. About 83 per cent of the washed eggs proceeding from infected mice hatched within one hour after brought under brilliant illumination, as compared to 31 per cent of others placed in a corner of the room (5 to 20 foot candles) and 18 per cent of those kept in the dark at room temperature or in the incubator at 37°C. Tests using human material gave similar results. Exposure of the eggs to a brilliant light is recommended when large numbers of free miracidia are desired within a specific limit of time.

33. *The occurrence of an Adult Holostome (Trematoda: Cyathocotylidae) in the Intestine of a Fish.* R. M. Cable and Winona B. Vernberg, Purdue University.

Channel catfish, *Ictalurus punctatus,* taken from the Wabash River at Lafayette, Indiana, have been found to serve as the natural definitive host of an undescribed species of *Cyathocotyloides.* The incidence of infection was aproximately 25 per cent in fish weighing at least one pound but much less in smaller specimens. The largest number of worms recovered from a single host was 19 which varied from small, immature specimens to several fully developed adults with numerous eggs. Two furcocercariae of the Vivax type occur in snails in the river. Since one is known to be the larva of *Linstowiella szidati,* the other may be the cercaria of *Cyathocotyloides,* a genus closely related to *Linstowiella.*

34. *Mucoid Glands in* Fasciola hepatica *Cercariae.* Francis J. Kruidenier, University of Illinois, Urbana, Illinois.

Amply confirmed classical descriptions of cercariae of *Fasciola hepatica* differentiate two types of unicellular, cystogenous glands. Thomas (1883) implied that at least one of these produces a "mucous substance"; this has been accepted in current literature. Thionin techniques do not demonstrate metachromasy for these glands in emerged cercariae or in developing cercariae dissected from snails.

A third type of unicellular gland, transitory in developing cercariae and highly metachromatic in thionin, contains presumptive mucoid secretions. They are distributed singly in the

subcuticula of the dorsal surface and along the dorso-lateral border of the body and in pairs along the length of the tail. Their numerous processes become reduced in proportion to the accumulation of cell secretions but are never obliterated before the discharge of these irregularly stellate glands. Cercariae are well differentiated preceding the appearance of these glands; glands in the tail develop later and disappear earlier than those in the body, discharging from cercariae still retained in rediae. Glands are not visible in the bodies of heavily stained, emerged cercariae, but discharge and are presumably used during the sojourn of cercariae in snail tissues, subsequent to their emergence from rediae.

These mucoid glands appear to be analogous to others demonstrable in numerous cercariae, resembling most closely those glands in the monostome group with which they may prove to be homologous.

35. Eimeria meleagrimitis *Tyzzer, 1929 in the Turkey.* Philip A. Hawkins, Michigan State College, East Lansing, Michigan.

This species is the most pathogenic of the coccidia occurring in the turkey, and experimentally produces a high mortality in poults two to three weeks of age. It is found throughout the small intestine and to a lesser extent in the rectum, but has not been found to localize in the cecum. It is most numerous in the middle third of the small intestine. The organism is found superficial to the nucleus of the epithelial cell, with none having been observed in the crpyts. The first asexual generation is completed two and one half to three days after infection. The second generation is completed about four days after infection and is asexual. The third generation is predominantly sexual and oocysts are passed in the feces towards the end of the fifth day of infection. A fourth generation, initiated by scattered third generation merozoites, occurs. However, the course of this infection in the tissues has not been followed past the eighth day after the administration of oocysts.

The oocyst has produced four sporoblasts in 18 to 20 hours after passage in the feces. At this time the small globular inclusion bodies may be seen, there being one to three present. Sporulation is not completed until two days after passage when maintained at room temperature.

The histopathological picture presented by this infection is remarkable. Five days after infection the epithelium from the tips of the villi, particularly in the middle third of the small intestine, has been lost. Only the intact basement membrane separates the lamina propria from the lumen of the intestine. Large dilated capillaries are noted in direct contact with the membrane, yet no hemorrhage is observed. In severe infections no parasites are seen in the middle third of the small intestine due to the loss of epithelium. In birds that survive, the regeneration of epithelium is very rapid.

36. *Echinococcosis in Lebanon and Its Incidence in Animal Hosts.* Alan C. Pipkin, University of Arkansas Medical School, Emile Rizk and Jirair Balikian, American University Medical School, Beirut, Lebanon.

The records of the American University Hospital at Beirut, Lebanon, show that over 100 patients infected with *Echinococcus granulosus* were admitted from 1931 through 1938. This is a direct reflection of the incidence of infection of dogs with the adult worm, and an indirect indication of the incidence of larval stages in animal intermediate hosts. Of 237 stray dogs autopsied, 32.9 per cent harbored adult hydatid worms, about 10 per cent with massive infections. To estimate the reservoir from which canine hosts were infected, a survey of slaughtered cows, camels, and sheep, was undertaken. Examination of 514 beef carcasses revealed that 48.05 per cent had indications of the infection, but only 36.43 per cent of these bore cysts still containing live scolices. The cysts were confined to the lungs in 55.87 per cent of the infected beeves, whereas 12.55 per cent showed liver involvement only. About 30 per cent of the infected animals showed cysts in both liver and lung, and 2.02 per cent of these had cysts in the heart muscle as well. Multilocular cysts were more prevalent in beef than unilocular cysts. Of 34 camel carcasses, 64.7 per cent had cysts in the viscera and 54.5 per cent of the infected animals contained still viable scolices. Sheep, commonly considered the animal most accessible to dogs on the range, showed an incidence of only 6.6 per cent infection in 500 carcasses, but 62 per cent of the sheep showing cyst-walls also contained live scolices.

37. *A Natural Mammalian Final Host* (Sorex *sp.*) *for an Acuariid Nematode.* Jack D. Tiner, Department of Zoology, University of Illinois, Champaign, and Robert Rausch, Alaska Health Service, Anchorage, Alaska.

Thirty shrews were examined at Juneau, Alaska, and 15 were found to be infected with a nematode which resembled *Dispharynx* sp. Infections ranged from one to nine worms per

host animal. Infected whole animals, sections of tissue from the stomach showing imbedded worms, and specimens of the nematode were prepared for demonstration.

38. *The Food of* Cyathostomum (*Nematoda: Strongylidae*) *Adults.* Norman D. Levine, University of Illinois, Urbana, Ill.

There is so much black pigment in the intestinal wall of *Cyathostomum* sp., one of the small strongyles of horses, that the intestinal contents cannot be seen. When the nematodes are fixed, treated with hydrogen peroxide to decolorize the pigment and render the cuticle permeable to resinous mounting media, and stained with hematoxylin, their intestines are seen to contain many *Cycloposthium* spp. (Protozoa: Oligotricha), a normal inhabitant of the horse colon. It seems possible that the adults of *Cyathostomum* do not feed on their host animal, but live on the protozoa and perhaps the bacteria in its large intestine.

39. *Sporocyst Generations of* Postharmostomum laruei *McIntosh* (*Trematoda: Brachylaemidae*). Martin J. Ulmer, University of Michigan, Ann Arbor, Mich.

Contrary to accepted ideas regarding the life cycle of *Brachylaeminae,* researches in progress show presence of mother and daughter sporocysts, the latter giving rise to cercariae. Results reported are based on feeding experiments using laboratory-reared *Anguispira alternata.* Ten days after exposure to eggs, small rounded mother sporocysts measuring 0.2–0.4 mm. with spherical or oval projections arising from a central core appear in connective tissues of the liver, near the digestive tract. 3 to 4-week mother sporocysts with slightly motile branches contain daughter sporocyst embryos floating free in the lumen. Increasing greatly in size, the mother sporocyst ramifies throughout the connective tissues of the liver, occasionally into the kidney, and at 7 weeks contains numerous daughter sporocyst embryos, some of which are oval and possess a lumen. They exhibit no structures characteristic of cercariae. At 7 or 8 weeks, elongate branches (2 mm.) of mother sporocyst move actively throughout connective tissues of liver and discharge young daughters through terminal birth pores. Daughter sporocysts develop rapidly and contain well organized cercariae at 10 weeks when sporocyst may be but 0.5 mm. long. Cercariae leave sporocysts via terminal birth pores and begin to leave snails at 12 weeks. Laboratory infections have been maintained for more than a year. Infestation by daughter sporocysts is massive, affecting liver, portions of mantle and kidney. More than two generations of sporocysts have not been found.

40. *An Anomalous Tapeworm Strobila Showing Reversal of the Proglottids.* Kathel B. Kerr, Dr. Salsbury's Laboratories, Charles City, Iowa.

Upon examining tapeworms removed from some cull chickens one strobila was found, a portion of which exhibited a complete double reversal of the proglottids. In all, 12 proglottids were involved. At the site of the primary reversal there is a ring of tissue giving the general impression of a greatly compressed proglottid. Where the proglottids again reverse to their normal position the segment is bulbous and presents the appearance of having been injured. Between the points at which the reversals took place the proglottids appear to have a normal structure except for their reversed position. The scolex was lost, so it is impossible to determine the species. The worm is undoubtedly of the genus *Raillietina.*

41. *The Eyelid Lesion Appearing in Chicks Infected with* Plasmodium lophurae. Elery R. Becker, Iowa State College, Ames, Iowa.

The eyelid lesion appeared in chicks on a ration that was composed of about 80 per cent corn, oats, and wheat products, but not in chicks on a ration with 15 per cent soybean oil meal and linseed oil meal. Neither did it appear in chicks on various commercial rations. The progress of the development of the lesion from the incipient stages first noted on the fourth to sixth day to the loss of the lower eyelid, and the regeneration of a new eye covering that may in certain cases completely overgrow the eye will be depicted. The lesion seems to be a more acute form of the one described by Norris and Ringrose from chicks suffering from pantothenic acid or biotin deficiency, because it was prevented by supplementing the ration with these vitamins, either together or separately.

42. *Dicrocoelid Trematodes from the Gorilla.* Horace W. Stunkard, New York University.

A young gorilla, which died in Bronx Park, New York City, was heavily infected with trematodes, located chiefly in the pancreas and identified tentatively as *Eurytrema brumpti* Railliet, Henry and Joyeux (Bull. Soc. Path. Exot., 5: 833–837; 1912). The present account appears to be the second report of this species, so far found only in African primates.

43. *Observations with the Phase Microscope on the Malaria Parasite* Plasmodium lophurae *and on Its Extracellular Development* in vitro. WILLIAM TRAGER, Rockefeller Institute, Princeton, N. J.

Photomicrographs will show the appearance of *P. lophurae* as seen with the phase microscope in freshly drawn infected duck blood. Especially conspicuous are the round, sharply outlined vacuoles which appear a pale pink in blood films stained with Giemsa. Nuclear material is barely visible in some of the larger segmenting parasites. While the normal living parasites within the host cell have a relatively bright appearance, those which have been extruded into the serum as a result of pressure on the coverslip rapidly become dark and dense. Such parasites, however, still appear normal when stained. If the parasites are removed from their host red cell by means of a hemolytic anti-serum many of them at first retain their normal appearance as seen by phase microscopy. If such parasites were then placed in a suitable jelled medium containing a concentrated duck erythrocyte extract, some of them survived and underwent development during a 2-day period of incubation *in vitro* at 40° C. Photomicrographs will show the appearance of such extracellular parasites as seen in the living state with phase microscopy and as seen in stained films. From these it will be apparent that not only did the extracellular parasites undergo segmentation but in addition some of the merozoites developed further into young trophozoites.

44. *Infection of the Immature White Mouse with the Avian Malaria Parasite,* Plasmodium lophurae. R. BARCLAY MCGHEE, Rockefeller Institute, Princeton, N. J.

Plasmodium lophurae parasitizes mouse erythrocytes when these are introduced into the blood stream of an infected chick embryo. Attempts to infect adult mice with this parasite were unsuccessful. Inoculation of a large number of parasites from an infected chick embryo produced infection in mice inoculated at less than one day of age. Initial infections reached a peak of 500 per 10,000 erythrocytes, and parasites could be found 8 days after parasite introduction. Alternate embryo-mouse infections indicated some adaptation, since peaks of 1100 per 10,000 erythrocytes were attained on the 3rd or 4th day. Parasitemias, characterized by declining peaks, were produced in three direct mouse passages. The asexual cycle in the mouse was 36 hours long and was more synchronous than in the embryo. The parasites in infected mice were similar to those in chicks in their general morphology, but differed slightly in their location in the red cell, their staining reactions, and the number of merozoites produced.

45. *The Mouth Parts of* Liponyssus bacoti *Hirst.* FLORA E. GORIROSSI. The University of Texas, Medical Branch, Galveston, Texas.

The first detailed description of the capitulum of this mite and its attached appendages is given. Special emphasis is placed on the modifications of the exoskeletal covering of the basis capituli as well as a description of the eight appendages of the mouth—the two dorsolateral prolongations, two maxillae, two labral stylets, hypopharynx and labrum—as they appear when individually isolated and in transverse and longitudinal sections. A possible relationship of these parts to each other and with the pharyngeal orifice is suggested. A general comparsion of the mouth parts of *Liponyssus bacoti* with those of *Laelaps echidninus* and *Ornithodorus moubata* is included. The technics used in the killing, preserving and mounting mites are discussed. This work was supported by Research Contract N8onr 61,000 of the Office of Naval Research under Dr. J. Allen Scott.

46. *Proof of the Direct Action of an Antibiotic on* Entamoeba histolytica. WILLIAM BALAMUTH AND MORGAN M. BRENT, Northwestern University, Evanston, Illinois.

During the past five years various antibiotics have been tested against *Entamoeba histolytica,* often with conflicting results. The greatest source of error rests in separating the sought-for direct action on the amoebae from an indirect action due to susceptibility of the essential bacterial associates. This problem has been solved in recent experiments employing prodigiosin, a pyrrole derivative extracted as a red pigment from *Serratia marcescens.*

Most of the tests involved monobacterial cultures of the NRS strain of *E. histolytica* and *Aerobacter aerogenes* grown in aqueous egg-yolk infusion plus rice starch. The concentration of prodigiosin ranged from $1:2 \times 10^{-5}$ to $1:2 \times 10^{-7}$. Runs were conducted either in 10 ml. or 35 ml. total volume. Bacterial and amoebic populations were traced for several days, as well as changes in pH and oxidation-reduction potentials.

The following general results were noted: The bacteria multiplied in all concentrations of prodigiosin, in the highest concentration reaching 300×10^6 per cu. mm. at 48 hours' exposure. No amoebae were found in the highest concentration after the first eight hours; very few survived in $1:2 \times 10^{-6}$; while fair growth occurred in $1:2 \times 10^{-7}$. The oxidation-

reduction potentials of all cultures ranged between E_h –150 to –300 mv. through the first 72 hours.

This is believed to provide the first published record of direct, specific action by an antibiotic against *E. histolytica* at significantly low concentrations. (This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health.)

47. *Experimental Infection of Guinea Pigs with* Endamoeba histolytica. D. Jane Taylor, Joseph Greenberg and G. Robert Coatney, National Institutes of Health, Bethesda, Maryland.

Amoebiasis has been produced in young guinea pigs by the intracaecal inoculation of trophozoites of *Endamoeba histolytica.* The infectivity rate among 194 animals was 58 per cent; with certain dietary modifications it has been possible to infect 100 per cent of the animals. Mortality rate of infected animals was 97 per cent. The mean day of death was 12.4 days after inoculation.

Symptoms of the infection are weight loss, diarrhea with demonstrable trophozoites, ulceration and other pathological changes of the cecal and colonic wall. Ulcers have been found to penetrate the submucosa.

48. *Further Studies on the Factor that Spares Erythrocytes from Phagocytosis and its Possible Significance in Malarial Infection.* Elery R. Becker, Alice A. Marousek, Dorwin Byrd, and Thomas Schwink, Iowa State College, Ames, Iowa.

It has been reported that duck plasma has the property of retarding the removal of duck erythrocytes parasitized with *Plasmodium lophurae* from the circulation of young chicks when the plasma is injected intravenously into the chick about two hours before the erythrocytes are injected. It is now shown that the serum has the same property as the plasma. That the effect is not attributable simply to a temporary increase in the total blood protein is shown by the ineffectiveness of injecting comparable amounts of chicken, rat, or human plasma. Neither is there any evidence that the sparing effect is due to blocking, toxic action, or haemolysis. The possible role of the factor that produces the effect in determining the course of the *lophurae*-infection will be discussed.

49. *The Antimalarial Activity of the Plasma of Certain Adult Ducks against* Plasmodium lophurae. William Trager and R. Barclay McGhee, Rockfeller Institute, Princeton, N. J.

It has been shown previously that the plasma of adult chickens, when injected into young chicks infected with *P. lophurae,* depresses the parasitemia. In similar experiments with ducks it was possible to test the effect of plasma from each individual adult duck on the course of infection in 5 one-week-old ducklings. The plasmas from about one fourth of the ducks which were tested markedly reduced the parasitemia in the treated ducklings. When the adult ducks from which these effective plasmas had been obtained were subsquently inoculated with *P. lophurae* they developed exceptionally light infections. The ducks which yielded plasmas having no effect when injected into infected ducklings uniformly developed severe and often fatal infections when they were later inoculated with parasites. Thus the plasma of those ducks having a relative natural resistance to *P. lophurae* contained a material capable of exerting an antimalarial effect when transferred to young fully susceptible ducklings. This method of passive transfer permits the study over a period of time of factors affecting the natural resistance of the individual animals, since the extent of this resistance can be determined without having to infect the animal.

50. *The Incidence of Blood Parasites in Liberia.* Martin D. Young, National Institutes of Health, U. S. Public Health Service, Columbia, S. C.

In a country-wide survey of Liberia in 1948, thick and thin blood smears were made of 10,128 persons. Microfilariae, principally *W. bancrofti,* were found in 84 smears (0.83 per cent). Trypanosomes were found in 5 instances (0.05 per cent). Malaria was found in 3,104 smears (30.65 per cent). The epidemiology of these parasites is discussed.

51. *The Parasitemia in Experimental Toxoplasmosis.* Leon Jacobs and Frances E. Jones, Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Maryland.

The parasitemia in animals infected with *Toxoplasma gondii* has been measured by the technique of blood inoculation. Mice, rabbits, rats, chicks, and pigeons infected with known numbers of toxoplasmas were studied. Acutely infected mice and rabbits showed a progressive increase in parasitemia to a high level several days before death; 0.5 ml of a 1:1,000 dilution of blood from these animals produced infections in mice. Mice, rats, and one rabbit with chronic

infections showed a low parasitemia sporadically. Of 5 pigeons infected with 5,000 toxoplasmas, only one died; the others showed no symptoms. From the first to fourth week after infection, these pigeons showed a parasitemia at least equal to that in acutely infected rodents; after 4 weeks, the blood was consistently negative. Eight-day-old chicks infected with 1,500 toxoplasmas showed a somewhat lower parasitemia from the fourth to seventeenth day. Thereafter the blood was negative but chicks sacrificed 2 months after infection showed an enlarged spleen and their tissues were infective to mice.

Blood smears from a rabbit at peak parasitemia showed toxoplasmas free in the plasma. Separation of blood elements by albumin flotation failed to produce a concentration of toxoplasmas, indicating that the parasites are not necessarily found only in white cells, as believed by some authors.

The data suggest that bloodsucking arthropods may be involved in transmission of *Toxoplasma,* and that bird ectoparasites merit particular attention because of the prolonged parasitemia in avian infections. The data have already been of value in determining the most propitious time for allowing possible vectors to feed on donor animals.

52. *Exoerythrocytic Forms in Relation to Paludrine Administration in Pigeons Infected with* Plasmodium relictum. W. B. Redmond and E. L. Fincher, Emory University.

Examinations of brain, bone marrow, lung, liver and spleen have revealed the presence of exoerythrocytic forms in pigeons infected with the 1P strains of *Plasmodium relictum.* Following blood inoculations the E. E. forms are found most abundantly 4 to 8 days following the disappearance of parasites from the blood. Approximately 25 per cent of the birds showed E. E. forms.

A strain of *P. relictum* which had been made paludrine resistant by subjecting it to noncurative doses through numerous transfers showed a much greater percentage of E. E. forms.

A series of 9 consecutive infections treated with 0.3 mg. paludrine daily injected intravenously for varying periods of 8 to 12 days developed parasitemias that were both higher and longer than normal. All birds except one in this series have shown E. E. forms.

A similar series consisting of 14 birds treated with 1 mg. paludrine daily administered orally have developed very slight parasitemias and only one bird has shown E. E. forms .

No examinations or E. E. forms were made until after the resistance had developed in the first series and no resistance has developed so far in any of the other series in which E. E. forms have been found. For this reason no correlation of the factors responsible for the development of resistance with the appearance of E. E. forms is possible. Under certain conditions, such as small doses of paludrine administered intravenously, there is a high correlation of the drug administration and the incidence of E. E. forms found.

53. *The Antimalarial Activity of Aureomycin against* Plasmodium gallinaceum *in the Chick.* G. Robert Coatney, Joseph Greenberg, W. Clark Cooper and Helen Louise Trembley. National Institutes of Health, Bethesda, Maryland.

During the routine testing of certain antibiotics for antimalarial activity, aureomycin was found active against *P. gallinaceum* in the chick. Against erythrocytic parasites (A-1 test) aureomycin reduced parasitemia with an effectiveness equal to about one-fourth that of quinine. In tests for causal prophylaxis (A-2 tests) the drug was able to prevent infection. In tests against late exoerythrocytic forms aureomycin was able to prevent death, a property previously exhibited only by certain 8-aminoquino-lines, chlorguanide (paludrine) and sulfadiazine.

54. *Respiratory Organs of Chiggers.* G. W. Wharton, Department of Zoology, Duke University, Durham, North Carolina.

André (1943) first reported stigmata and tracheae in a chigger, *Acomatacarus paradoxa.* Womersley (1945) independently discovered these organs in *Acomatacarus longipes* and later suggested that they were characteristic of the subfamily Leeuwenhoekiinae. Wharton (1947) found stigmata and tracheae to be characteristic of the Apoloniinae and inferred, by using this characteristic to differentiate between the Apoloniinae and the Trombiculinae, that stigmata and tracheae would not be found in the Trombiculinae. Recently a new species of *Neoschöngastia* was found that possessed tracheae and stigmata. At present stigmata and tracheae are known to occur in the following genera of the Trombiculidae: Leeuwenhoekiinae *Leeuwenhoekia, Acomatacarus, Hannemania, Odontacarus,* and *Whartonia;* Apoloniiae *Apolonia* and *Womersia;* Trombiculinae *Neoschöngastia* (one of the species only); and Walchiinae none. The spotty distribution of stigmata and tracheae in the family indicates that they are adaptive modifications and have little phylogenetic significance, or that the present system of classification of the trombiculids is artificial.

55. *Studies on the Pyrethrum Synergist, Piperonyl Butoxide, and Their Bearing on Use of These Two Insecticides in the Control of Arthropod Pests.* Merritt P. Sarles, U. S. Industrial Chemicals, Inc., Baltimore, Maryland.

The organic chemical, piperonyl butoxide (3,4-methylenedioxy-6-propylbenzyl butyl diethylene glycol ether), has been the subject of toxicity studies for four years. The initial studies demonstrating that insecticide solutions containing practical concentrations of this chemical were relatively nontoxic and nonirritating involved tests for primary eye and skin irritation, sensitization, and acute oral and subcutaneous toxicity (Amer. Jour. Trop. Med., (1949) 29: 151). Later, extensive chronic toxicity tests were made on dogs and rats. Dogs fed piperonyl butoxide by capsule six days a week for one year survived dosages of up to 0.1 ml./kg./day (total doses of up to 530 ml.). Rats fed from weaning to two years of age on treated food containing 0.01% and 0.1% of piperonyl butoxide, or 0.1% plus 0.2% pyrethrins, differed insignificantly from control rats in respect to food consumption, weight gain, reproduction (three successive generations raised on treated food), blood picture, and mortality, and showed no evidence of gross or histological pathology. The chronic-toxicity threshold was only reached for rats at the relatively high concentration of 1.0% of piperonyl butoxide in the food, which caused an approximately 25% reduction in food consumption, with related decrease in weight gain and poorer reproduction, and an increased mortality in the second year, although some rats survived the full two-year period, during which they ingested approximately their own weight of the chemical. Piperonyl butoxide was found to be one of the least toxic insecticides and therefore of special value for the control of arthropod pests in situations where safety is of primary importance.

56. *The Behavior of the Adult Female Snake Mite,* Ophionyssus natricis, *in Response to Directed Light and the Effects of Body Weight on these Reactions.* Joseph H. Camin, The Ohio State University, Columbus, Ohio.

While studying the biology of the snake mite, it was observed that the adult females exhibit varying degrees of negative photo-taxis. Experiment showed that the more heavily engorged the parasites were, the more definite was this reaction. The peculiar movements of the first pair of legs suggested they might be involved in the reception of light stimulus. Removal and masking of segments of these legs led to the conclusion that the photo-receptors are located on the pulvilli and microscopic examination has tentatively confirmed this. The first pair of legs, waving from side to side in front of the mite, orients the animal in the direction of its own shadow and away from the light source. The more heavily engorged the parasite is, the greater is the shadow it casts; and the larger the shadow, the more definite is the negative response to light. Further experiments revealed that the rate of locomotion of these animals is inversely proportional to the body weight and it is believed that this also plays an important part in the orientation or lack of orientation away from a directed light beam.

The net results of these responses in combination with other reactions, not yet analysed, are that the unengorged female mite gets onto the snake host, becomes engorged with blood, and is then driven off the host and into dark crevices, where it deposits its eggs.

57. *Temperature, Oribatid Mites and the Development of* Monoecocestus (*Cestoda: Anoplocephalidae*). Reino S. Freeman, University of Minnesota.

Studies have been initiated on the effect of temperature on the larval development of the porcupine tapeworms *Monoecocestus americanus* and *M. variabilis* in the oribatid mite *Liacarus* sp. Controlled temperatures of 25°, 20°, 15°, 10° and 5° C. were used. After feeding the tapeworm eggs, fully formed cysticercoids were found within 45 days at 25° C., 52 days at 20° C. and 82 days at 15° C. Fully formed cysticercoids have not as yet been found in mites kept at 10° and 5° C., although very immature larvae have been found as late as 127 days after feeding in mites kept at 5° C. The average size of cysticercoids at different temperatures was found to vary, with the maximum size at 15° C. and minimum size at 25° C. Measurements of cysticercoids from a limited number of naturally infected *Liacarus* sp. suggests that natural development takes place at temperatures under 20° C. To supplement this, it was found that the mean leaf litter temperature during the month of August, 1949, in a habitat where *Liacarus* sp. occur, was 17.1° C. with a standard deviation of 1.7.

58. *Preliminary Studies on the Intermediary Metabolism of the Cestode* Hymenolepis diminuta. Clark P. Read, the Rice Institute, Houston, Texas.

Preliminary studies have included chemical identification and estimation of the acid-soluble phosphorylated compounds present in "resting" tissue, identification of specific enzymes known to be associated with phosphorylative glycolysis in other organisms, and characterization of the phosphatase activity in homogenates of cestode tissue.

Fractionation of acid-soluble phosphorus compounds was carried out by barium-alcohol separation. The fractions were studied by appropriate chemical procedures. Identification of some of the compounds was verified by an isotopic dilution method utilizing radio-active phosphorus (P^{32}). The substances identified and estimated in the worm tissues are essentially similar to those reported to be concerned with glycolysis in other animals. An exception is the apparent lack of phosphagen (arginine phosphate or creatine phosphate). This is being investigated further.

Specific enzymes associated with glycolysis were studied by partial purification from extracts of cestode tissue and subsequent incubation with appropriate substrates. Enzymes identified were phosphorylase, aldolase, phosphohexoisomerase, and 3-phosphoglyceraldehyde dehydrogenase.

The phosphatase activity of tissue homogenates after the addition of different substrates was studied. The activity with all substrates was enhanced by the addition of magnesium ion and inhibited by the addition of calcium ion. The optimum pH with all substrates was 7.6. This is strikingly similar to the pH optimum of rat intestinal mucosa phosphatase which differs from that of other rat tissues in showing the greatest activity at pH 7.4 with all substrates tested. The activity is greatest in the anterior quarter of the worm (measured linearly) and decreases posteriorly in the second and third quarters. In the posterior quarter the phosphatase activity again increases, presumably due to developing eggs. There is evidence that the tissues contain a substance which nullifies the magnesium activation.

59. *Observations on the Nervous System of the Cestodes.* Nathan W. Riser, Department of Zoology, University of Pennsylvania, Philadelphia, Penna.

The major commissures between the two laterial nerve trunks in the cestode strobila, lie in the region of the connection between proglottids. They occur behind the anastomoses of the excretory trunks in forms where such anastomoses occur. Most workers have described the commissures as lying in the posterior part of the proglottids. It is frequently difficult to ascertain where a proglottid begins and ends in the strobila, but in the attached proglottids of *Phyllobothrium tumidum* Linton and *Scyphophyllideum giganteum* (van Ben.), and in the free proglottids of the hyperapolytic tetrarhynch *Lacistorhynchus tenuis* (van Ben.) the commissures can readily be demonstrated in the anterior ends of the proglottids.

60. *Interrlations of* Diphyllobothrium *with Fish-Eating Birds of Northern Lake Michigan.* Lyell J. Thomas, University of Illinois, Urbana, Illinois.

Investigations of *Diphyllobothrium* infection of gulls and terns from the Beaver Island archipelago, Lake Michigan since 1936 disclose the following interrelations with lake herring, copepods, and young birds.

Mayfly and caddisfly emergents during June and July coincide with the appearance of ciscoes in deep water near the surface and the development of young birds which are fed herring by the parents. The *Diphyllobothrium* is shown experimentally to mature in young laboratory hatched and reared gulls and Caspian terns in 3½ to 4 weeks after which time they are usually shed. During this period the young are feathered and learn to fly. The tapeworm develops in birds in the rookeries similarly and is shed in waters about the islands in late July and August in great numbers. The circuitous water currents about the islands of northern Lake Michigan tend to confine the segments to that area. During August and September the herring retire to deep water and both old and young birds disperse in numbers to inland lakes. The herring spawn in the shoals about the islands in October and November and hatch in the spring. Experiments with *Diphyllobothrium oblongatum* eggs indicate they can withstand freezing for at least one month and at 2° C. for five years. Thus a continual source of infection is maintained for the first intermediate host, *Diaptomus oregonensis* which is one of the chief foods of the herring. The procercoid in the copepod becomes a plerocercoid in fish in from one to two months in cysts on stomach and mesenteries.

61. *Infections of Chickens with Cysticercoids and Infected Intermediate Hosts of* Raillietina Cesticillus. Kathel B. Kerr, Dr. Salsbury's Laboratories, Charles City, Iowa.

A study was made comparing the rate and degree of infection resulting in New Hampshire chickens 7 to 13 weeks of age when infected either with cysticercoids removed from the intermediate host or the infected intermediate host, *Tribolium sp.* (probably *T. confusum*). Ten cultures of beetles were used, eight of which showed infections of 85 per cent or better. The degree of infection in the beetles ranged from 1 to 47 cysticercoids, with 58 per cent of the beetles harboring from 1 to 10 cysticercoids.

Forty of 47 birds, or 85 per cent, each infected with 41 to 78 cysticercoids (40 received

50 cysticercoids each) harbored worms at autopsy. All 60 of the birds given infected triboliums estimated as harboring 51 to 87 cysticercoids were infected at autopsy.

The percentage development (number worms/number cysticercoids administered) from the cysticercoid infections was 26.6 and from the beetle infections 33.6 The greatest difference between the two groups was that some of the birds given the infected beetles harbored more worms.

The data indicate that birds given infected beetles develop sufficiently constant infections to serve for routine testing of substances for anthelmintic activity and that infection with cysticercoids should probably be used in more critical tests.

62. *A Plate Method of Screening Chemicals as Molluscicides.* Donald B. McMullen, 406th Med. Gen. Lab., and Univ. of Okla. Med. School.

Since submerging amphibious snails in a series of dilutions of potential molluscicides has certain disadvantages another method of bringing them in contact with chemicals was devised. It was found that *O. nosophora* would live for at least 8 months on moist filter paper. Ten snails in the center of a piece of filter paper, covering the bottom of a Petri dish (150mm), made a suitable unit for testing various dilutions. Two cc of liquid completely wetted the filter paper so this amount was used in each dilution, *i.e.,* 1 : 1000, 2000, 4000, 10,000, 20,000, etc. to 160,000. Thirty-nine chemicals and proprietary compounds were tested. Water, xylol, alcohol and other solvents were tried. In some series the test solutions were added with snails present. In others the solvent was allowed to dry, then water and snails were added. Notes were taken at intervals of 1, 2, 6, 24, 48, 72 and 96 hours. If snails had moved they were centered and water was added to keep the paper moist. On the fourth day the snails were washed and placed in water for several hours. All snails that did not show activity were crushed. This technique made it possible to observe irritancy, toxicity, residual effect, and effect of different methods of application, under conditions similar to those found when chemicals were applied in the field. With some of the more promising chemicals dilutions of 1, 5, 10, 20, etc. to 100 ppm were used. These gave curves typical of toxicological experiments. Field tests had been made with many of the chemicals before this test was devised. There was a good correlation between field and laboratory tests. All chemicals with a 50% end point less than 1 : 20,000 could have been eliminated.

63. *Seasonal Studies on* Schistosoma japonicum *in the Intermediate Host,* Oncomelania nosophora. Donald B. McMullen, 406th Med. Gen. Lab., Tokyo, Japan and Univ. of Okla. Med. Sch., Oklahoma City, Oklahoma, T. Endo-Itabashi, 406th Med. Gen. Lab., S. Seto, 406th Med. Gen. Lab., S. Komiyama, Yamanashi Health Dept., Kofu, Japan, Paul R. Stone, 406th Med. Gen. Lab.

Four collecting stations were established in Yamanashi, Prefecture, Japan for seasonal studies of *S. japonicum* in *O. nosophora.* Over a two-year period monthly collections of more than 2000 snails were examined. Comparison of data from the stations shows considerable variation, but in general the following points are of interest. The snails acquired infections at anytime they were active, but most of them were picked up in May–July. A smaller number of young infections appeared in the autumn. The highest rates of infection were found in July and August. The incidence and stage of development remained more or less constant during the hibernation period. After a hot, dry summer the incidence during the hibernation period was low and nearly all infections were young. After a wet, cool summer the incidence was higher and about half the infections carried through the winter were mature. As a result of this winter carry-over the number of infections capable of producing cercariae during the spring farming season in 1949, was more than five times that of the previous year. A large proportion of the infections acquired early in the summer died out by early fall. Most of the infections lived 3–4 months but some, the number varying with the season, lived for 10–12 months. More than half this period is spent in the immature stage or in hibernation.

64. *Immunologic Studies.* I. *Experiments with Bird and Human Schistosomes.* G. W. Hunter, III, L. S. Ritchie, W. D. Tigertt, S. Lin, C. Pan and H. Tanabe, 406th Medical General Laboratory, Tokyo, Japan.

A series of experiments were set up which were designed to test possible immunity (1) to reinfections by (a) a bird schistosome or (b) *S. japonicum;* (2) to initial exposures to one species followed by successive exposures to the second species in an attempt to see if one produced sensitivity and/or immunity in the other.

Both mice and rabbits were utilized. Reactions were not uniform. Thirty-six (36) white mice exposed twice to *S. japonicum* gave no visible evidence of any dermatological reaction.

However, five (5) of these gave a detectable reaction when exposed to cercariae of a bird schistosome which produces a marked dermatitis in man.

Experiments in which 13 rabbits were exposed twice initially to bird schistosomes yielded only one sensitive animal. A second exposure within 1–5 days yielded three more reactions. Approximately one month later 5 of these rabbits were exposed to cercariae of *S. japonicum* but no reaction occurred. A like number exposed to cercariae of the bird schistosome all gave marked reactions which were confirmed histologically. Two control rabbits gave lesser reaction to an initial exposure.

Protective ointments including dimethyl and dibutyl phtallate and copper oleate gave some promise in preliminary experiments under both laboratory and field conditions.

65. *Protection Experiments with Copper Oleate Ointment against Schistosomiasis.* E. KAUFMAN, G. W. HUNTER, III, AND C. PAN, 406th Medical General Laboratory, Tokyo, Japan.

During 1948 and 1949 the effectiveness of copper oleate was tested as a protective agent against the penetration of cercariae of *S. japonicum.* While this ointment was designed to protect man against schistosomiasis, of necessity mouse protection tests were the basis for this study. The problems included the question of how successfully it could be used as a skin ointment and the mode of anti-cercarial action both *in vitro* and *in vivo.*

Copper oleate is a semi-solid, dark blue, sticky substance which is insoluble in water but freely soluble in ether and other fat solvents. At body temperature it is a sticky, highly viscid semi-liquid. It is most easily applied to the skin in an ether solution. The ether evaporates in five or ten minutes leaving a thin, sticky film which is difficult or impossible to wipe off completely; soap and water will not remove it.

Schistosome cercariae tested *in vitro* lose their motility after 15 to 30 minutes contact with a copper oleate film. Soluble materials seems to play no part in this effect inasmuch as water "saturated" with copper oleate has no untoward effect on the cercariae. Besides the "chemical" action cited above, it was presumed that the oleate might also act as a mechanical barrier. Mice were used *in vivo* testing of the material. The back of the animal was completely shaved and covered with a film of copper oleate. The prepared surface was then superimposed over a water bath consisting of a paraffin-lined watch glass set into a wooden block. One set of control animals was shaved and exposed without the protection of the oleate film. Another control involved use of oleate film without exposure to cercariae; this procedure was used in an attempt to detect any deleterious effect resulting from the chemical and procedures employed. Application and removal of the oleate was timed, so that the ether used as a solvent would not have a chance to effect the cercariae.

The copper oleate ointment apparently gives good protection, since 34 out of 36 mice exposed were uninfected at autopsy. The two positive mice yielded only seven (2 and 5 respectively) *S. japonicum* in contrast to the unprotected controls where up to 99 parasites were recovered from a single animal. Apparently the protection is not due to a mechanical effect alone as other control mice protected by a paraffin-vaseline or pure vaseline mixture became infected. Toxicity studies which were carried on to determine the possible effects of copper oleate on the survival of mice gave no indication of any marked deleterious effect. It would appear, therefore, that copper oleate has considerable value in protecting against the penetration of *S. japonicum* cercariae in mice.

66. *Multiplication of Germinal Cells in the Rediae of* Clinostomum marginatum. W. W. CORT, The Johns Hopkins University; D. J. AMEEL, Kansas State College; ANNE VAN DER WOUDE, University of Michigan.

The germinal cells in the rediae of *Clinostomum marginatum* are in groups of two to five free in the body cavity; only occasionally were single germinal cells seen. In small redial embryos only the germinal cell groups are present in the primitive body cavity. In later stages up to mature rediae they are scattered in different parts of the body cavity mixed with the embryos. These groups of germinal cells must be dividing rapidly since large numbers of embryos are produced. This distribution of germinal material is very different from that in all other rediae we have studied, which have included representatives of the order Fasciolatoidea and of the families Hemiuridae, Lissorchiidae, Allocreadiidae, Troglotrematidae and Heterophyidae. In the rediae belonging to these groups more or less persistent germinal masses are present at the posterior end of the body cavity which serve as centers of multiplication of germinal cells. *C. marginatum* belongs to a different order of the digenetic trematodes from any of these forms, being related to the schistosomes and strigeids. Perhaps the germinal cell groups in the rediae of this species are the prototypes of the complicated floating germinal

masses of the strigeids. In fact they are almost exactly like the early stages of the strigeid germinal masses in very small embryos of daughter sporocysts. This mechanism for multiplication in the rediae of *C. marginatum* is very effective since this species appears to produce more cercariae from a single infection than any other trematode species we have studied.

This investigation was supported (in part) by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

67. *Parasites of northwest Wisconsin fishes.* Jacob H. Fischthal, Triple Cities College of Syracuse University, Endicott, New York.

In a survey of fish parasites during the three year period from 1944 through 1946 from 124 lakes and streams in northwest Wisconsin 4,532 fishes belonging to 61 different species and subspecies were examined and 4,186 or 92.4 per cent were infected with at least one species of parasite. This percentage of infection is relatively high in comparison with surveys conducted in other regions of the United States and Canada. Of the 4,532 fishes examined, 34.4 per cent were from streams. The fishes from streams were 85.3 per cent parasitized, while those from lakes were 96.1 per cent infected. A separate record of the number of fish infected with each parasite from each water as well as the intensity of infection was maintained for each species of fish examined. Soft water lakes showed only light parasitism with a dearth of especially trematodes and acanthocephalans. As the hardness of the water increased the incidence and intensity of parasitism increased accordingly. The fishes from the cold water trout streams showed a lighter incidence and intensity of infection than exhibited by those from warm water streams. The larval parasites were most frequently encountered. Those most often observed were *Clinostomum marginatum, Diplostomulum* spp., glochidia, *Neascus* spp., *Posthodiplostomum minimum, Proteocephalus ambloplites, Spiroxys* sp., *Leptorhynchoides thecatus,* and *Pomphorhynchus bulbocolli.* Also encountered frequently in immature or adult stages were the latter two acanthocephalans, Gyrodactyloidea, *Proteocephalus pearsei, Camallanus oxycephalus, Contracaecum* spp., *Spinitectus carolini* and *gracilis,* and the Myxosporidia.

68. Clonorchiasis: Report of a Case in a Caucasian Patient Observed in Boston. Donald L. Augustine, Harvard School of Public Health, Boston, Mass., and Howard J. Isenberg, Beth Israel Hospital, Boston, Mass.

This appears to be the fifth case of clonorchiasis recently observed among the white race in the Western Hemisphere. All of these cases acquired the infection within the same given period in Shanghai, China, and under similar circumstances. They represent a specific example of global dissemination of a regional disease by mass movement of populations during World War II.

69. *The Relationship of Male Worms to the Sexual Development of Female* Schistosoma mansoni. Donald V. Moore, Tamarath K. Yolles, and Henry E. Meleney, Department of Preventive Medicine, New York University College of Medicine, New York.

In the absence of male worms, female *Schistosoma mansoni* worms are incapable of developing to sexual maturity. When male worms are introduced into the same host these female worms complete their arrested sexual development. Experiments were performed to determine at what stage in the development of the added male worms maturation of the females would occur. Albino mice harboring only female worms for 11 weeks were exposed to cercariae known to produce male worms. Autopsies were performed at half-week intervals and the degree of development of both male and female worms determined. No further development of the female worms was noted until 5 weeks after the exposure of the mice to male cercariae, when some of the male worms had reached maturity. All of both sexes were mature 1½ weeks later. In a group of control mice exposed simultaneously to male and female cercariae, some of the female worms were mature 5½ weeks after exposure and all were mature 1½ weeks later.

Additional experiments were performed in an attempt to produce sexual development of the female worms by (1) injection of a suspension of desiccated male worms, (2) injection of testosterone, and (3) implantation of living mature male worms into the peritoneal cavity of mice harboring only female worms. No evidence of sexual development of the female worms was observed, indicating that sexual maturation of female *S. mansoni* requires intimate contact with mature males.

70. *The Fate of Dermatitis-Producing Schistosome Cercariae in Laboratory Animals.* Louis J. Olivier, Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Maryland.

In an effort to trace the fate of dermatitis-producing schistosomes, laboratory animals which had had no previous exposure to schistosome cercariae, were exposed by placing cercariae on the clipped skin. 21 albino mice were exposed to large numbers of cercariae of *Trichobilharzia stagnicolae*. No worms were recovered from the lungs or liver 1 to 7 days after exposure. Moreover, there was no other gross evidence of lung invasion by the worms. However, when 24 mice of the same strain were exposed to cercariae of *Trichobilharzia ocellata* (*Cercaria elvae*) the lungs of all but one bore conspicuous macroscopic lesions and worms were recovered from the lungs of 5. Worms and lesions were also observed in the lungs of 3 hamsters, 2 albino rabbits, and 1 rhesus monkey, and lesions but no worms in 9 hamsters, 8 guinea pigs, and 2 rhesus monkeys following exposure to the cercariae of *T. ocellata*. With the exception of the one mouse cited, no animal failed to develop pulmonary lesions following exposure to the cercariae. All the worms recovered from the lungs were readily identified as schistosomes. The worms were usually small and some were sluggish and apparently moribund; nevertheless, all showed evidence of morphological change following penetration. In some cases this consisted chiefly of loss of glandular material, slight enlargement of the gut, and dispersal of the eye-spot pigment. In other specimens, however, the gut was greatly enlarged and elongated and contained numerous, brown, refractile particles resembling the gut contents of mature schistosomes. One worm was recovered after 7 days in a hamster and one after 10 days in a rabbit. All the other worms were recovered 4 days or less after penetration.

71. *Partial Development of* Echinorhynchus coregoni *in* Hyalella azteca *and the Cellular Reaction of the Amphipod to the Parasite.* D. L. DeGiusti, Wayne University, Detroit, Michigan, The Catholic University of America, Washington, D. C., and the University of Michigan Biological Station, Cheboygan, Michigan.

Echinorhynchus coregoni is an acanthocephalan parasitic in the digestive tract of coregonid fish. Juveniles of this form have been found in the amphipod, *Pontoporeia hoyii*.

In the present work it was found that the eggs of this acanthocephalan will hatch when ingested by *Hyalella azteca*. The acanthor penetrates the amphipod gut wall and locates just beneath the serosa. In most instances the acanthor ceases to develop, is walled, and is destroyed by cellular reaction of the host. The host reaction consists of giant cells which coalesce to form a syncytium.

A small per cent of the infected amphipods do not overcome the infection. In these hosts the acanthor enters the acanthella stage and has been followed through eight days of development with no apparant signs of injury.

The mechanism of liberation of the acanthor from its embryonic membranes was observed within the amphipod intestine.

The acanthor of *Echinorhynchus coregoni* has a prominent rostellum armed with blade-like hooks. Its body is covered with fixed spines, large at the anterior extremity and progressively decreasing in size posteriorly.

Hyalella azteca appears to be an abnormal intermediate host for *Echinorhynchus coregoni*, but it serves as a good tool both for the study of the cellular reaction of the amphipod to an acanthocephalan parasite and as a means of liberating the acanthor from its embryonic membranes for study.

72. *A Gregarine Parasite in the Amphipod,* Hyalella azteca. Peter J. Batten and D. L. DeGiusti, Wayne University, Detroit, Michigan and The University of Michigan Biological Station, Cheboygan, Michigan.

Hyalella azteca collected from Douglas Lake, Michigan, have been found to harbor within their intestine a cephaline gregarine. The sporonts are broadly oval-shaped and average twenty microns in length. A circle of deeply-staining granules at the anterior end of the protomerite suggests the presence of an epimerite during an earlier stage. A syzygy consists of two individuals. Cysts averaging thirty microns in diameter are formed in the hindgut. Their development from the gametocyst through spore duct formation has been observed. Twenty-four hours after a cyst is formed three thickened circular areas representing the basal disks of the spore ducts appear on its surface. Growth of the spore tubes begins at these basal disks and progresses inward towards the center of the cyst. Work is at present being conducted to experimentally complete this life cycle.

This form agrees with Kamm's description of the genus, *Gregarina*. Since *Hyalella azteca* is a new host record for any gregarine, this parasite is proposed as a new species, *Gregarina hyalellae*. The measurements given above indicate that *Gregarina hyalellae* is the smallest described gregarine.

73. *Failure to Demonstrate Precipitins in Dogs Infected with* Endamoeba histolytica. J. C. Swartzwelder and G. R. Muller, Louisiana State University School of Medicine, New Orleans, Louisiana.

Wagener (1924) reported positive precipitin tests with sera from kittens infected with *E. histolytica.* The antigen was an aqueous extract of scrapings of intestinal lesions. Positive reactions were most frequently observed in animals which had discharged amebae in the stool 8 or more days. In the present study, precipitin tests were run on 29 dogs. Thirteen dogs had active amebic infections of several weeks to several months duration. Trophozoites were demonstrable in dysenteric aspirates of the colon at the time the tests were conducted. Sixteen dogs were negative for amebae. Three of these animals had not been inoculated with *E. histolytica* and 13 had recovered from experimental infections and were free of demonstrable infection for periods ranging from one to three months. The antigen employed in these studies was obtained from Hynson, Westcott and Dunning, and is ordinarily used for complement-fixation reactions. No positive precipitin reactions were obtained with sera from any of the dogs. The tests were performed with both untreated and inactivated sera. Tests were read promptly upon completion, after standing at room temperature, after incubation, after refrigeration and following centrifugation. These results on precipitin studies in amebic infected dogs are reported since they are markedly at variance with the experience of Wagener with kittens. These represent incidental observations during a project on immunity in amebiasis supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service.

74. *Cerebral Hydatid Infection. Report of a Case Which Recovered Following Surgical Removal of the Parasite.* G. C. Anderson (deceased) and J. C. Swartzwelder, Louisiana State University School of Medicine, New Orleans, Louisiana.

A white male patient, 23 years of age, native of Louisiana, was admitted for hospitalization with complaints of severe persistent headache, drowsiness and lethargy. He was semicomatose on admission. Physical, neurological and ophthalmic examinations, and ventriculograms gave evidence of increased spinal fluid pressure, bilateral limitation of right visual fields and a markedly dilated left ventricle, which pointed to presence of a tumor or cyst. There was no X-ray or physical evidence of cystic involvement of either the lungs or liver. Air or dye injected into either ventricle failed to pass to the other ventricle, indicating lack of communication. While withdrawing the needle from the left ventricle following the above exploratory surgical procedure, an adherent cyst-like membrane appeared. This was removed by gentle traction. The material measured eight cm. at its largest diameter and contained five smaller cysts; the largest measured three cm. in diameter. The membrane removed proved to be that of an hydatid cyst. Diagnostic hooklets were also present in the accompanying fluid. Two weeks after removal of this hydatid material, a strongly positive intradermal reaction was elicited employing non-specific tapeworm antigen extract. The patient improved dramatically promptly following the above surgical procedure and was discharged three weeks later. Approximately eight years later, the patient had no recurrence of symptoms and was employed at manual labor. This case is reported since it is unique in that the patient recovered from an ordinarily fatal infection, cerebral hydatid disease.

75. *Culture Experiments on Intestinal Flagellates. V. Some Additional Longevity Records to September 1, 1949.* D. H. Wenrich University of Pennsylvania, Philadelphia, Pa.

Using previously described methods of prolonging the life of individual cultures by adding fluid (usually distilled water)and nutrients (usually powdered gastric mucin or Loeffler's dried blood serum) from time to time, certain tube cultures have been maintained for one to six years, depending upon date of origin. Samples are listed: 1) *Trichomonas batrachorum* from *Rana pipiens,* 6 years. (total age, 6 yrs., 11 mo.); 2) *T. wenyoni* from white rat, 4 yrs., 7+mo.; 3) *T. wenyoni* from hamster, 4 yrs., 2½ mo. (total age, 5 yrs., 5 mo.); 4) *T. prowazeki* from *Amphiuma means,* 3 yrs., 5½ mo.; 5) *T. augusta* from *Rana pipiens,* 2 yrs., ½ mo. (total age, 2 yrs., 3½ mo.); 6) *T. hominis* from man, 3 yrs., 4 mo.; 7) *T.* sp. from *Crotalus horridus,* 2 yrs., 1 mo., 8) *T.* sp. from *Thamnophis sirtalis,* 1 yr., 11 mo. (total age, 2 yrs., 2 mo.); 9) *T.* sp. from *Python reticulatus,* 1 yr., 5 mo. (total age, 1 yr., 6 mo.); 10) *T.* sp. from *Sceloporus* sp., 1 yr., 2 mo.; 11) *T.* sp. from *Iguana, iguana,* 1 yr., 2 mo.; 12) *Trimitus parva* from *Triturus viridescens,* 2 yrs., 2 mo. (total age, 3 yrs., 5½ mo.); 13) *Monocercomonoides* sp. from tipulid larva, 4 yrs., 4 mo.; 14) *M.* sp. from Japanese beetle larva, 4 yrs., 4½ mo.

All cultures listed are originals except 1, 3, 5, 8, 9, and 12, which are transplants; total age of strain given in parentheses. Culture 6 has been maintained at 32–34° C.; all others have been at room temperature. All cultures listed were positive on September 1, 1949.

76. *Studies on Encystation of* Endamoeba histolytica. I. *Size of Inoculum, Rate of Multiplication and Density of Population Compared to Degree of Encystation.* Martha Grace Everritt, Tulane University.

Two strains of *E. histolytica* were compared quantitatively with respect to the influence of size of inoculum, rate of multiplication, and total density of population upon encystation. Inocula ranging from 100 to 50,000 amebae were subcultured from a starch-free medium to one containing rice starch. Inocula of 5,000 amebae and above produced significantly higher percentages of cysts than the smaller inocula. Encystation up to 40 per cent of the total population occurred with one strain, as compared with 10 per cent with the second strain. pH ranges were similar for the two strains. The rate of multiplication was suggested as of possible causative importance, since the strain which encysted in high percentages multiplied at a faster rate than the strain which encysted poorly. The rate of growth of the former strain was rather constant regardless of the size of inoculum, although the smaller inocula (less than 5,000) resulted in considerably fewer cysts and a smaller total population. From equal inocula and within the same period of time, the strain which encysted well reached a population level of over 2 million in 15 cc. of culture medium when 5,000 to 50,000 organisms were inoculated, while the other strains attained a population of less than one million under similar conditions. There was evidence of a more direct correlation between the density of population and the amount of encystation than the actual rate of multiplication. (Supported by a grant from the National Institutes of Health.)

77. *Studies on Encystation of* Endamoeba histolytica. II. *Influence of Density of Population vs. Rate of Multiplication on Encystation.* Martha Grace Everritt, Tulane University.

This study was primarily designed to discover the relative influence of population density and rate of multiplication on encystation. Most of the work was carried out with one strain. The procedure consisted of subculturing trophozoites from a starch-free environment to a measured volume of medium containing starch. The inoculum in most cases consisted of 25,000 amoebae. The principle observations made were as follows: (1) Agitation of a culture at frequent intervals increased the rate of multiplication and the total population, but to a considerable extent inhibited encystation; (2) after the onset of encystation, agitation of a culture was followed by progressive diminution in the number of cysts present; (3) in the cultures not recently agitated, the increase in the percentage of cysts was proportional to the logarithmic increase in the size of the population; (4) in comparing growth and encystation in flasks and tubes of various sizes, the size of the population necessary to stimulate encystation appeared to be directly proportional to the size of the vessel used. The assumed importance of the rate of multiplication, as observed by other workers and during the early part of the present study seems to be due to its relation to production of dense populations which stimulate encystation. (Supported by a grant from the National Institutes of Health.)

78. *Action of Neomycin on Protozoa.* Sachiko J. Ishihara and Oscar Felsenfeld, Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Illinois.

Neomycin in concentrations lower than 100 units per ml. inhibited the growth of *Endamoeba histolytica* strains, while approximately 550 units were necessary to check the multiplication of most of the tested *Trichomonas vaginalis* cultures. Trypanosomes required as much as 1,000 units per ml. culture medium. Animal experiments gave results which were in accordance with the outcome of the *in vitro* tests.

79. *An Unusual Strain of* Dientamoeba fragilis. Viola Mae Young, Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Illinois.

Repeated stool examinations revealed *D. fragilis* containing one or more red blood cells in an adult patient suffering from clinical amebiasis of Craig type IV. The findings were confirmed by culture and hematoxylin stained slides. It is believed that this is the first instance in which *D. fragilis* engulfing red blood cells was found in the feces.

80. *The Effect of Penicillin on the P strain of* Entamoeba histolytica *and Associated Bacterial Flora,* in vitro. Irving Pierce Delappe, Michigan State College, East Lansing, Michigan.

The growths of this strain of *Entamoeba histolytica* and its associated bacterial flora were correlated, with and without penicillin treatment (500 units per ml.). In the absence of penicillin there was an increase in bacterial and amoebic populations during the 18 hour period following subculture. During the same period, but in the presence of penicillin, there was a decrease in bacterial population and the amoebic population did not increase appreciably. If

these last tubes were subcultured and subjected to a second treatment of penicillin, bacterial growth decreased steadily and amoebae were not present from 24 hours on.

This indicates that one treatment with penicillin hampers the growth of the bacterial flora and the medium is conditioned very slowly for the amoebae. This brings about a prolonged lag phase in amoebic growth. When two treatments of penicillin are given, and the second treatment is given after subculturing at 18 hours, bacterial growth is so retarded that the amoebae expire before the medium can be conditioned properly for them.

Although the dependence of *Entamoeba histolytica* on its bacterial flora has been established incontrovertibly before this, it is of some interest to note the effect of penicillin at the above dosage level when it is adminstered in this particular time sequence. That the effect of penicillin is not exerted directly on the amoebae, *per se,* can be demonstrated by adding bacterial flora (grown apart from the amoebae) coincidentally with the second penicillin treatment (at 18 hours). When this is done, the amoebae do not disappear.

81. Entamoeba histolytica *Infections in Young Chicks.* M. J. Miller, Institute of Parasitology, Macdonald College, P.Q., Canada.

The results of intra-rectal inoculations of over 700 chicks up to 48 hours old has established the fact that trophozoites of *Entamoeba histolytica* will live and multiply in the caeca of chicks for a period of about five days at the end of which time they disappear. On several occasions infections were established in 96-hour-old chicks and, in one instance, a seven-day-old chick was infected. The infection was invariably confined to the caeca, and in only a few chicks were amoebae found in the large intestine below the caecum. At no time were amoebae seen in the small intestine. Infections have been established by oral inoculations of both cysts and trophozoites. In none of the infections was there any evidence of host tissue damage caused by the amoebae. Under conditions of the experiments carried out to date, the highest infection rate obtained was 50 per cent, although with some strains all attempts to induce infection failed. The bacterial complex accompanying the amoebae in culture influenced the infectibility of the amoebae, and while monobacterial cultures of amoebae with *Clostridium perfringens* as the accompanying bacterium was found to be one of the most suitable cultures for infecting chicks, this phase of the problem is incomplete and still under investigation.

82. Giardia *Infection in a Chinchilla.* Banner Bill Morgan, University of Wisconsin, Madison, Wisconsin.

A 13 months-old male South American chinchilla (*Chinchilla laniger*) was brought to the laboratory for post-mortem examination. The lungs showed hypostatic congestion with gross pneumonic involvement of the anterior lobe. Marked emaciation, edema of the heart and mesentery was present. Examination of the intestine revealed a mild congestion and a heavy infection of *Giardia* sp. They were most abundant in the jejunum and ileum. The trophozoites measured 11 to 20 microns in length by 6 to 10 microns in width. They could not be distinguished from *G. lamblia.* Oval cysts were 14.7 microns long by 8.82 microns in width. Spherical cysts measured from 9 to 11 microns in diameter. Not more than 4 nuclei were observed in any cyst. Motile trophozites failed to develop in 5 hamsters, 11 white mice, 3 rabbits and 5 guinea pigs fed *Giardia* cysts from the chinchilla.

83. *The Relationship Between Numbers of Adult* Trichinella spiralis *in the Small Intestine and the Precipitin Titer of Mice Given Various Test Infections.* James R. Hendricks, University of North Carolina.

Four experiments were performed with female mice, 6 weeks old. The 4 groups (10 mice each) of experimental mice were given 1, 2, 3, and 4 stimulating infections (200 larvae), respectively, at 21-day intervals, before they were given a challenging infection of the same size. The controls (6 mice) for each experiment were given only the challenging infection. All of the mice were bled and autopsied 5 days after the challenging infection. Counts were made of the number of adult worms in the small intestine of each mouse and the precipitin ring-test was performed with pooled sera from each group.

There was no significant difference in the number of worms recovered from the experimentals and controls of experiment 1 despite the presence of preciptins (titer of 1.14) in pooled sera of the experimentals.

In experiments 2, 3, and 4 the experimental mice harbored significantly fewer adult worms than their controls. Moreover, there was a progressive significant decrease in numbers recovered from the experimentals in each case. Associated with this resistance there was a progressive increase in antibody titer (1:64, 1:128, and 1:1024).

It appears, therefore, that there is a quantitative relationship in this resistance between

number of worms developing and the antibody titer. Further studies are planned in which both measurements will be used to compare the effects of various factors, such as dosage, time intervals in the infecting schedules, etc.

84. *Comparing in Mice the Percentage Development of* Trichinella spiralis *Larvae Obtained from a Recent and from an Old Infection in Rats.* James R. Hendricks, University of North Carolina.

Forty-six female mice, 6 weeks old, were divided equally into 2 groups. Those of Group I were given 300 active larvae from a rat infected 1 month previously and those of Group II were given the same number of active larvae from a rat infected 12 months previously. The steps followed to free the larvae from the rat muscle by artificial digestion and to infect the mice were identical for both groups. It is worth mentioning that in addition to the 300 active larvae present there was an average of 5, apparently dead, larvae in the 0.1cc dose given the mice of Group I and an average of 25 such larvae in the dose given the mice of Group II. This suggests that either more larvae from the old rat were dead prior to digestion or were less able to survive such treatment. All of the mice of both groups were killed 7 days after infection for counts of adults in the small intestine.

The mice of Group I showed a significantly higher percentage development (72.5) than those of Group II (56.9). It appears, therefore, that large numbers of the active larvae from the old rat were unable to establish in the small intestine. If this indicates a loss of infectivity, it would be important in certain studies on resistance to consider the source of the larvae used for infection.

85. *Tests in Mice to Determine the Relationship of Intestinal Emptying Time and Natural Resistance to Infection with Pig Ascarid.* John E. Larsh, Jr., University of North Carolina.

Three experiments were performed to establish the minimal infecting egg dose for female mice, two months old. There were, in all, 19 groups with an average of 5 mice each. Each group was given a different dose of eggs, ranging from 600 in group one to 30,000 in group 19. Only the mice of the last group showed larvae in pressed lung sections prepared 5 to 8 days after infection. Therefore, as compared with guinea pigs which were infected in a previous study with as few as 6600 eggs, the mice demonstrated a striking natural resistance.

A final experiment with females of the same age was carried out to determine whether the rapid intestinal emptying time of mice of this age is a factor in their striking resistance mentioned above. Sixteen mice were used, eight in the experimental group were injected with one per cent morphine sulphate to slow the emptying time (the drug's action was verified in additional controls). Then the 16 mice were each given 12,000 eggs. The controls, as expected from the above, showed no larvae in pressed lung sections; however, all but one of the drugged mice autopsied had numerous larvae in the lungs. This increased susceptibility of the drugged mice is best explained by their reduced intestinal emptying time, which presumably allowed greater numbers of eggs to hatch than in controls. Thus, the rapid intestinal emptying time of mice of this age probably is an important factor in their strong resistance to initial infection with pig *Ascaris,* as demonstrated earlier in natural resistance to *Hymenolepis.*

86. *Studies on the Life History of* Capillaria annulata *(Molin, 1858) Cram 1926.* Rex W. Allen, U. S. Bureau of Animal Industry.

Capillaria annulata, a nematode parasite of the upper digestive tract of poultry and many game birds, has a world-wide distribution and is of common occurrence in this country. Wehr (1936, North Am. Vet. 17: 18–20) ascertained that earthworms are the intermediate hosts, and he demonstrated transmission with *Eisenia foetida* and *Allolobophora caliginosa.* Present studies confirmed these findings and, in addition, demonstrated that *Lumbricus terrestris* may serve as an intermediate host.

Eggs of *Capillaria annulata* became fully embryonated in 32 days in a shallow layer of tap water held at room temperature. Unembryonated eggs held for 21 days either at 37° C. or at 4° to 6° C. did not develop appreciably during that time. However, when the same eggs were subsequently held at room temperature, development progressed at a rate approaching the normal. Embryonated eggs hatched *in vitro* when placed in an extract of intestinal contents of an earthworm. Examination of sections of an inoculated earthworm showed the longitudinal muscles to be the preferred site of the larvae. Development to a stage infective to the final host required between 14 and 21 days in *A. caliginosa* and between 21 and 28 days in *L. terrestris.* During development the larvae increased in size and changed morphologically. Development to the adult stage, as evidenced by first appearance of eggs in the feces of the final host, required between 19 and 25 days in turkeys and between 19 and 26 days in chickens.

87. Necator americanus *and* Ancylostoma duodenale *Infections in Puerto Rico.* José Oliver-González, José M. Dobal, and Carlos J. Thillet, School of Tropical Medicine, San Juan.

Sixty three convicts from the Insular Penitentiary, Guaynabo, Puerto Rico received tetrachlorethylene for the treatment of hookworm. The ages of the convicts varied from 19 to 61 years. They came from all sections of the island, and all evidence seems to indicate that the infections were acquired outside of the penitentiary. The doses of tetrachlorethylene administered varied from 2 to 3 cc., given on an empty stomach, followed one hour later by a saline purgative. All stools passed during the first 72 hours after the administration of the drug were collected and examined for adult worms.

Of the 63 individuals treated, 16 or 25.4 per cent had mixed infections of *Necator americanus* and *Ancylostoma duodenale.* The remaining 49 had infections of *Necator americanus* only.

88. *Diet of Hens and Development of* Ascaridia galli *in their Chicks.* A. C. Todd and M. F. Hansen, Kentucky Agricultural Experiment Station, Lexington.

An experiment designed to compare soybean meal and corn gluten meal as sources of protein in "animal protein factor" deficient diets for laying hens provided an opportunity to study the effects of varying diets for laying hens on the resistance of their offspring to parasitic infection. Six groups of New Hampshire hens were fed a corn gluten meal basal diet, or a soybean meal basal diet, or each of these diets supplemented with 5 per cent whitefish meal or 14 micrograms of Vitamin B_{12}. Chicks tested from the 6 groups of hens were all fed the same commercial starting and growing ration. Significant differences were found in percentage development and lengths of *A. galli* grown in the 6 groups of test chicks. Significant differences in growth of the 6 groups of chicks were also obtained, following uniform exposure to 50± infective eggs of the parasite.

89. *The Effect of Inhibitions, Intermediates and a Stimulant upon the Oxygen Consumption in* Ascaridia lineata. J. Christopher Mitchell, S. Milton Nabrit, Atlanta University, and B. F. Smith, Spelman College, Atlanta, Georgia.

A study of oxygen consumption of whole Ascaridia, freshly removed from the intestinal tract of the chicken was made in Barcroft-Warburg respirometers.

Sodium malonate, a succinic dehydrogenase inhibitor, did not block oxygen uptake. Fifty-eight per cent inhibition occurred in $\frac{M}{400}$ KCN. NaN_3 inhibited oxygen consumption twenty per cent at $\frac{M}{400}$ concentration and pH 5.2. Combined KCN–NaN_3 produced an additive inhibition of seventy-nine per cent. Ethyl urethane did not consistently produce inhibition. Sodium succinate was indifferent. Methylene blue stimulated oxygen uptake less than one per cent. Cytochrome *C* stimulated O_2 uptake twenty-five per cent. Nitrogen paralyzed KCN and NaN_3 inhibited worms in an hour. Paralysis in fresh worms occurred in 1½ hours in nitrogen.

We interpret these results to indicate that the enzymes used, after semi-anaerobic existence in the intestine, for oxygen uptake are essentially oxidases. They also suggest that oxygen debts incurred must be satisfied by O_2.

90. *An Iron Alum-Picric Acid-Hematoxylin Stain for Parasites in Tissues.* Morris Goldman, Communicable Disease Center, Atlanta.

A staining technique for tissues is presented which yields critical results comparable to those achieved by Heidenhain's iron hematoxylin method but which eliminates the troublesome step of destaining.

The following schedule may be used:

1. Remove paraffin from sections and hydrate in usual manner.
2. Mordant in 4% iron alum solution for 20 minutes or more at room temperature.
3. Wash in running water for 10 minutes.
4. Transfer to a 1:1 mixture of saturated aqueous picric acid and 0.1N HCl for 3 hours or more at 37° C.
5. Rinse in distilled water for 1 minute.
6. Stain in ripened 0.5% hematoxylin for 2 hours or more at 37° C.
7. Wash in running water for 30 minutes.
8. Dehydrate and mount in the usual manner.

Longer time periods for steps 2 and 4 do not effect final results very much; extended staining in hematoxylin may yield dense preparations but gross blackening does not occur.

The above procedure has given excellent results with *E. histolytica* in colon and *L. donovani* and *T. cruzi* in spleen, liver and heart fixed in formalin, Bouin's and Zenker's fixatives.

91. *The Effect of Pregnancy on the Natural Resistance of Mice to* Hymenolepis *Infection.* JOHN E. LARSH, JR., University of North Carolina.

Although previous work with mice shows that their natural resistance to this parasite is modified by various factors, as far as is known the effect of pregnancy has not been tested.

Only one experiment has been completed to date. Fourteen virgin female mice, 2.5 months old, were divided equally into experimental and control groups. The former were mated and after about 2 weeks of gestation had elapsed, they and the controls were each infected with 1000 *Hymenolepis* eggs. The experimentals harbored an average of about twice as many cysticercoids as the controls (67.7 and 31.2). Associated with the increased susceptibility of the experimentals was an average reduction in hemoglobin level, from 100% to 78% (Sahli). This relationship of reduced resistance to *Hymenolepis* and a well pronounced anemia also was observed in a previous study in old mice (5 to 13 months old) splenectomized when 2.25 months old. It would appear desirable, therefore, to continue the present study, and if additional experiments reveal similar results an attempt should be made to demonstrate the nature of this relationship. If this could be acocmplished, it would add to our knowledge of the operation of natural resistance to this parasite.

92. Cysticercus fasciolaris *in the Wild Rat.* KENNETH E. DYE, HAROLD M. KEMPLE AND WAYNE W. WANTLAND, Illinois Wesleyan University, Bloomington, Illinois.

A study of one hundred twenty-eight rats from the city dump area of Bloomington, Illinois was made to determine the incidence of *Cysticercus fasciolaris* and the effect of this parasite upon the liver tissue of the rat. The livers of all rats were carefully examined for cysts in various stages of development. One hundred seven rats were found to harbor one or more cysticerci which when fed to cats developed into the adult tapeworm *Taenia taeniaformis.* Cysts ranged in size from 2 to 9 mm. (greater diameter) and the largest number of cysts found in one liver was thirteen. Heavy leucocytic infiltration was observed in areas close to the thick fibrous wall of advanced cysts but no liver sarcomas sometimes associated with the cysticerous stage of this helminth were demonstrable. Microscopic examination of the diaphragms of the rats revealed no *Trichinella spiralis* larvae.

93. *Antibiotics as Bacteriostatic Agents for the Cultivation of Cestodes* In Vitro, W. MALCOLM REID AND JANET I. BOLES, Marine Biological Laboratory, Woods Hole, Mass., and Monmouth College, Monmouth, Ill.

Various commercially available antibiotics were used to prevent overgrowth of bacteria in culture media devised for the *in vitro* cultivation of *Hymenolepis diminuta* and *H. nana.* Best results were obtained, with a maximum survival time of 10 days, by various combinations of penicillin (1,852 units/cc), streptomycin (1,000 μg/cc), and chloromycetin (800 μg/cc). The nutrient medium consisted of Locke's solution combined with 1/3 strength of a nutrient medium consisting of 19 amino acids and 23 other constituents (Medium VI, Steele, Sauberlich, Reynolds, and Baumann. Jour. Biol. Chem. 177: 533–544. 1949). Aureomycin (500 μg/cc), in combination with penicillin was too toxic for use as a bacteriostatic agent. Although the nutrient medium was sterilized, antibiotics were relied upon to prevent bacterial growth of contaminants introduced by equipment, other solutions, and cestodes.

The antibiotics effectively inhibited bacteria, but all tubes were quickly overgrown with yeasts. Since streptomycin, penicillin and chloromycetin were not toxic, they might be useful in controlling bacterial contamination if used in combination with some other technique in which more antiseptic measures were employed.

94. *Experimental Infections with Cysticerci of* Taenia taeniaeformis *in Laboratory Animals.* EVERETT L. SCHILLER, University of Wisconsin, Madison, Wisconsin.

Known numbers of viable eggs or terminal proglottids of *Taenia taeniaeformis* were fed to 150 white mice, 3 rabbits, 10 hamsters and 4 white-footed mice. Wide variations occurred in the size and number of cysticerci produced in the livers of white mice, regardless of the number of eggs ingested. The degree of infection had no significant effect on the growth rate of white mice as determined by weight. Large numbers of cysticerci produced no apparent outward symptoms. The presence of 1 or more cysticerci in the liver protected the host against a subsequent infection. Less than 96 hours are required for migration of the onchosphere from the small intestine to the liver. The average number of cysticerci per mouse fed approximately 50 eggs each was 13.3 at the end of 42 days. The young of infected females showed no prenatal infection. Cysticerci did not develop in hamsters, white-footed mice or rabbits. Controls including 20 white mice, 3 hamsters, 2 rabbits and 2 white-footed mice were negative. Subsequent experiments were carried out on 20 additional rabbits (10 young and 10 aged) plus 10 controls.

Active terminal proglottids of *T. taeniaeformis* ranging from 2 to 13 in number were administered. All of the rabbits were negative for cysticerci.

95. *Gross Parasitism in a Young Raccoon.* Everett L. Schiller and Banner Bill Morgan, University of Wisconsin, Madison, Wisconsin.

A young emaciated female raccoon (*Procyon lotor*) weighing only 2253 grams was taken in southern Wisconsin. It had been brought to the laboratory for a diagnosis of rabies because it had attacked a trapper. The animal was negative for rabies (stain for Negri bodies and injections of brain tissue into white mice). However, observations revealed a tremendous infestation with lice (*Trichodectes crassus*), which were distributed over the entire body. Preliminary random counts suggested a total well over 30,000 lice. All stages of the life cycle were present. The masses of pearly-white eggs at the base of the hairs masked the appearance of the skin. Two immature ticks (*Dermacentor variabilis*) were removed from the back. Approximately 341 tapeworms (*Oochoristica procyonis* Chandler, 1942) were found in the duodenum. This is believed to be the only other record of the occurrence of this tapeworm since the species was described. *Physaloptera rara* occurred in the stomach and small intestine; 105 worms were obtained.

96. *Fluctuation in the Glycogen Content of the Cestode* Hymenolepis diminuta, Clark P. Read, the Rice Institute, Houston, Texas.

Chemical determinations of the glycogen content of *H. diminuta* at different times of the day show that there is a fluctuation in the glycogen level which may be correlated with the feeding of the host. Starvation of the host for twenty hours results in a reduction of the glycogen to about 10% of that in worms from fed animals. This is similar to the results obtained by Reid with *Raillietina.* However, in rats fed only sodium lactate during a twenty hour period the glycogen content of the worms was reduced only to about 40% of the level in worms from fully fed animals. Presumably, this indicates that lactate may be utilized by this tapeworm and furnishes direct evidence, not hitherto available, that some aerobic metabolism is carried on *in vivo.* The possible relation of a small, but necessary, amount of oxidative metabolism to the crowding effect in cestode infections will be discussed in some detail elsewhere.

97. *Experimental Tapeworm* (Moniezia expansa) *Infections in Young Lambs.* K. C. Kates and A. Goldberg, U. S. Bureau of Animal Industry, Beltsville, Md.

M. expansa cysticercoids, obtained from oribatid mites by dissection, were fed to 16 lambs. The number fed per lamb varied from 121 to 411. Infections were established in 14 of the 16 lambs. Seven of the infected lambs were killed and autopsied 30 to 34 days after infection. A total of 1,120 cysticercoids were administered and 463 tapeworms (41.3% of cysticercoids fed) recovered. One lamb harbored only 9 *Moniezia* and the other six lambs from 53 to 114. Complete tapeworms recovered varied in length from 9 inches to 14 feet, and in development from immature to gravid specimens. The quantity of strobilae from individual lambs varied from about a pint to a full quart. The other seven infected lambs were killed and autopsied 62 to 68 days after feeding cysticercoids. Feces of six of these lambs became negative from the 51st to 68th day and no tapeworms were recovered at autopsy; however, considerable quantities of tapeworm material were recovered from the feces during patency. One lamb, autopsied on the 65th day after infection, contained 68 tapeworms, amounting to over a pint of strobilae.

Tapeworm infected lambs showed no clinical evidence of infection. No significant weight differences between infected and control lambs were noted. It may be tentatively concluded that heavy experimental *Moniezia* infections in lambs are either non-pathogenic or the pathogenicity is of a low order. These observations do not eliminate the possibility that under conditions other than those that prevailed in this experiment tapeworms may produce injury to sheep.

98. Quickella (*Family Succineidae*), *a New Host for Sporocysts of* Leucochloridium (*Trematoda: Brachylaemidae*) *in Southeastern Michigan.* Irving G. Kagan, Dept. of Zool., University of Michigan, Ann Arbor, Michigan.

Leucochloridium sporocysts resembling *L. problematicum* Magath (1920) and the red-brown sporocyst of Woodhead (1935) were found 5 times in Michigan during 1948–1949 in snails of the genus *Quickella* (Family *Succineidae*). These snails are usually mistaken for *Succinea avara* on the basis of shell characters but are readily identified on autopsy by the presence of a huge appendix on the penal sac. One infected snail was collected at Ann Arbor, Michigan; 4 from Lake Erie marshes near Monroe, Michigan. Comparison of metacercariae from broodsacs taken from *Quickella sp.* show no morphological differences from red-brown broodsacs found in *Oxyloma retusa,* formerly *Succinea retusa.* In the United States, sporocysts of *Leucochloridium*

have been reported from 2 species of *Succineidae, Succinea ovalis* and *Oxyloma retusa.* This is the first record of such an infection in the genus *Quickella* and the first reported occurrence of *Quickella* for the midwestern part of the United States.

99. *The Presence of* Metorchis conjunctus *in Maine.* Marvin C. Meyer, University of Maine, Orono, Maine.

Among animals autopsied by students three were found parasitized with *Metorchis conjunctus,* which apparently constitutes the first record of its occurrence in the northeastern United States. During April, 1949, one dog from Bangor, and two racoons from Swan Island, were positive for this species of Opisthorchiidae.

Specimens were recovered from the gall bladder and biliary ducts; 12 from the dog, more than 100 from the first racoon, and 50 from the second. In the latter *Procyon lotor,* the only one examined personally, the liver was turgid, with various constrictions and several yellowish areas. When opened, a viscid, brownish-green, fluke-laden exudate covered the area.

Reports of *M. conjunctus* in Canada, synonymy and life cycle were discussed by Cameron (1944. Canad. J. Res. *22*: 6–16). It is found in man, dog, red fox, mink, racoon and cat, occurring generally throughout eastern Canada. Metacercariae are obtained from the common sucker, *Catostomus commersonnii.*

This, however, may not constitute the first record of its occurrence in the United States. Hung (1926. Proc. U. S. Nat. Mus. No. 2627. 69(1) : 1–2) described *Parametorchis noveboracensis* from the gall bladder of a New York cat, and Price (1929. *Ibid.* No. 2809. 76 (12) : 1–5) described *P. intermedius* from the gall bladder of a Wisconsin silver fox. Cameron regarded these species as identical with *M. conjunctus,* and Price (1940. Proc. Helminthol. Soc. Washington. 7 : 1–13) removed them from *Parametorchis* but failed to state to which genus they belong or pass on their validity. The present records and those of Hung and Price definitely establish another opisthorchiid in the United States.

100. *Parasites of the Ranidae (Amphibia). XVIII.* A. C. Walton, Knox College.

The annotated catalog of the parasites of the genus *Rana* continues the list of hosts and their parasites as follows:—80. *Rana spinosa* (Fr. Indo-China)—*Cosmocercella neveri* (Nematoda); and *Balantidium* sp? of Wichtermann, 1934, *Nyctotherus cheni,* and Opalinids of Wichtermann, 1934 (Protozoa). 81. *Rana stenocephala* (Brazil)—*Megalodiscus temperatus* (Trematoda). 82. *Rana sylvatica* (Canada)—*Microfilaria ranae-sylvaticae* (Nematoda); and *Dactylosoma sylvatica* (Protozoa). 82a. *R. sylvatica* (U. S. A.)—*Agamascaris odontocephala, Cosmocercoides dukae, Oswaldocruzia pipiens,* and *Rhabdias ranae* (Nematoda); *Gorgoderina attenuata,* and gorgoderid cysts of Rankin, 1945 (Trematoda); Plerocercoid cysts and larvae of Rankin, 1945 (Cestoda); and *Haptophrya michiganensis,? Nyctotherus cordiformis, Opalina larvarum* (in tadpoles), *O. virguloidea,* and *Trichomonas augusta* (Protozoa). 82b. *R. syl. cantabrigensis* (U. S. A.)—*Cepedea cantabrigensis* (Protozoa). 82c. *R. syl. latiremis* (U. S. A.)—*Balantidium* sp? of Metcalf, 1923, and *Cepedea cantabrigensis* (Protozoa). 83. *Rana tagoi* (Japan)—*Acanthocephalus lucidus* (Acanthocephala). 84. *Rana temporalis* (Ceylon)—*Opalina ranarum orbiculata* (Protozoa). 85. *Rana temporaria=R. platyrrhinus* (Europe)—*Agamonema ranae-temporariae, Ag.* sp? of v. Linstow, 1909, *Aplectana acuminata, A. schneideri, Cosmocerca commutata, C. minuscula, C. ornata, "Filaria" jubae, "F." rubella, "F."* sp? of v. Linstow, 1889, *Gordius aquaticus, G. setigera, G.* sp? of Leydig, 1953, *Icosiella neglecta, Myoryctes weismanni, Oswaldocruzia bialata, O. filiformis, Oxysomatium brevicaudatum, O. longispiculum, Rhabdias bufonis, R. microoris,* and *R. rubrovenosa* (Nematoda).

101. *Parasites of the Ranidae (Amphibia). XIX.* A. C. Walton, Knox College.

85 (con.). *Rana temporaria* (Europe)—larval and adult *Allocreadium angusticolle, Brachycoelium salamandrae, Cephalogonimus retusus, Cercaria cambrensis I* (in tadpoles), larval *Codonocephalus urniger,* larval *Distoma gyrini,* larval *Echinoparyphium recurvatum,* larval *Euryhelmis squamula* (in tadpoles), *Gorgodera cygnoides, G. microovata orientalis, G. pagenstecheri, G. varsoviensis, Gorgoderina vitelliloba, Haematoloechus similis, H. variegatus, Halipegus ovocaudatus, Haplometra cylindracea, Lecithopyge ranae, L. rastellum, L. rast. cylindriforme, ? L. rast. subulatum, L. subulatum, Lepoderma mentulatum, Opisthioglyphe endoloba, Opisthodiscus subclavatus, Pleurogenes claviger,* larval and adult *P. medians* (metacercariae also in tadpoles), *Polystoma integerrimum, P. uncinatum, Prosotocus confusus,* larval *Tetracotyle crystallina,* and larval *Tylodelphys excavata* (Trematoda); larval *Diphyllobothrium erinacei, Nematotaenia dispar,* and *"Taenia"* sp? of Giebel, 1866 (Cestoda); *Acanthocephalus ranae* (Acanthocephala); *Amoeba limax* (types 3 & 4 of Epshstein. 1926), *Balantidium duodeni, B. elongatum, B. entozoon, B. nucleus, Cepedea dimidiata, C.* sp? of Wright, 1930, *Chlamydophrys stercorea, Copromonas sub-*

tilis, Crithidia gerridis (experimentally cultivated), *Dactylosoma ranarum, Eimeria neglecta, E. ranae, E. ranarum, Entamoeba histolytica* (types 1 & 2 of Epshstein, 1926), *E. invadens, E. ranarum, Eutrichomastix batrachorum, Glugea danilewskyi, Hexamita intestinalis, Isospora lieberkühni, Lankesterella minima* (=*L. monilis,* = *ranarum*), *Leptomonas jaculum, Leptotheca ohlmacheri, L. ranae, Myxosoma ranae, Nematopsis temporariae, Nyctotherus cordiformis, Opalina ranarum, O. ran. arvalis, O. ran. cinctoidea, O. ran. truncata, Protoopalina intestinalis, Retortamonas dobelli, Trepomonas agilis* ? (probably *Giardia agilis*), *Trichomonas batrachorum, Trimitus parvus, Trypanosoma inopinatum, T. rotatorium, T. rota, nana,* and *T.* spp? of Danilewsky, 1885, and of Laveran & Mesnil, 1912 (Protozoa) ; *Lucilia bufonivora* (Diptera) ; *Basidiobolus ranarum,* and *Dermocystidium ranae* (Phycomycetes) ; and *Borrelia omelianskyi* (n. comb. for *Spirochaeta omelianskyi* Yakamoff, 1925) (Bacteria).

102. *Parasites of the Ranidae (Amphibia). XX.* A. C. Walton, Knox College.

85a. *Rana temporaria* (introduced into Japan)—*Oswaldocruzia yezoensis* (Nematoda) ; and *Oligobdella orientalis,* and *O. tagoi* (Hirudinea). 85b. *Rana temporaria ornativentris* (Japan, Korea)—*Oswaldocruzia bialata* (Nematoda) ; *Polystoma ozakii* (Trematoda) ; and *Acanthocephalus lucidus* (Acanthocephala). 85c. *Rana temporaria parvipalmata* (Europe-France)—*Opalina ranarum, O. ran. parvipalmata,* and *Trichomonas batrachorum* (Protozoa). 86. *Rana terrestris* = *R. arvalis* = *R. oxyrrhina* (Europe)—*Haplometra cylindracea* (Trematoda) ; *Balantidium entozoon, Isospora neos, Nyctotherus cordiformis, Opalina ranarum, O. ran. arvalis, Trypanosoma mega,* and *T. rotatorium* (Protozoa) ; and *Lucilia bufonivora* (Diptera). 87. *Rana theileri* (Africa)—*Trypanosoma nelspruitense,* and *T. rotatorium* (Protozoa). 88. *Rana tibetana* (China-Tibet)—*Omeia hoepplii* (Nematoda). 89. *Rana tigerina* (S. E. Asia)—published elsewhere. 90. *Rana trinodis* (Africa)—*Trypanosoma rotatorium* (Protozoa). 91. *Rana tuberculosa* (Africa)—*Trypanosoma mega,* and *T. rotatorium* (Protozoa). 92. *Rana verrucosa* (India)—*Opalina* sp? of Metcalf, 1940 (Protozoa). 93. *Rana virescens* (Japan)—*Opisthodiscus subclavatus* (Trematoda). 94. *Rana viridis* (Turkestan)—"Microfilaria" of Yakimov & Shokov, 1916 (Nematoda) ; and *Halipegus ovocaudatus* (Trematoda). 95. *Rana vittigera* = *R. cancrivora* (Philippines)—*Oxysomatium punctatum* (Nematoda) ; *Glypthelmins staffordi,* and *Pleurogenes taylori* (Trematoda) ; and *Cepedea longa macronucleata,* and *C.* sp? of Metcalf, 1940 (Protozoa). 96. *Rana* spp? (Brazil)—*Agamonema ranae,* and *Oxysomatium membranosa* (Nematoda) ; and larval *Distoma repandum* (Trematoda). 97. *Rana* spp? (Europe)—*Capillaria costacruzi,* ? *"Enterobius vermicularis,"* and *"Oxyuris" bidentata* (Nematoda) ; larval *Distoma agamo* (Trematoda) ; and *Amoeba oblonga, Spironucleus elegans,* and *Trichomonas* sp? of Pittaluga, 1923 (Protozoa). 98. *Rana* sp? (Philippines)—*Diplodiscus amphichrus* (Trematoda). 99. *Rana* spp? (U. S. A.)—*Eimeria pylori,* and *Myxidium serotinum* (Protozoa). 100. *Rana* spp? tadpoles (Europe)—*Hemiclepsis marginata* (Mollusca). 101. "Ranid" (Br. E. Africa)—*Amphibiophilus acanthocirratus* (Nematoda).

103. *The incidence of parasites of* Rattus norvegicus *in Wisconsin.* Everett L. Schiller and Banner Bill Morgan, University of Wisconsin, Madison, Wisconsin.

Eighty rats (*Rattus norvegicus*) collected over a period of a little more than a year (1947–1948), from a city dump area near Madison, Wisconsin were examined for parasites. Twenty of the rats, 12 of which were sexually immature and weighing less than 80 grams, were free of parasites. The remaining 60 rats all exhibited varying degrees of parasitism. The following results were obtained. Fifty of the 80 rats (62%) were infected with *Nippostrongylus muris.* The number of worms ranged from 1 to 241. *Trichosomoides crassicauda* was recovered from the urinary bladder of 20 rats (25%). Only 2 rats (2.5%) were parasitized with *Heterakis spumosa* while 4 animals (5%) harbored *Capillaria* sp. *Trichinella spiralis* was not encountered in this survey. *Hymenolepis nana* was the most prevalent cestode being recovered from 30 rats (37.5%). The number of worms varied from 1 to 267 per animal. Only 1 rat (1.2%) harbored *H. diminuta.* One species of trematode was encountered; 2 rats (2.5%) were infected with *Fibricola cratera.* The tropical rat flea (*Xenopsylla cheopis*) was removed from 1 rat while 4 were infected with *Liponyssus bacoti.* Severe kidney lesions characteristic of those described for *Leptospira icterohemorrhagiae* were observed in 4 rats, however, silver stained sections of these tissues failed to reveal this organism.

104. *A Check List of Parasites of Horses in Kentucky,* L. S. Olsen, A. C. Todd, and M. F. Hansen, Kentucky Agricultural Experiment Station, Lexington.

Cataloging of parasites of horses was initiated in 1947. A total of 15 genera and 26 species have been recorded as follows: *Anoplocephala perfoliata, A. magna, Parascaris equorum, Oxyuris equi, Setaria equina, Strongylus edentatus, S. equinus, S. vulgaris, Tridontophorus*

brevicauda, T. tenuicollis, Gyalocephalus capitatus, Cylicodontophorus bicoronatus, Cyathostomum labratum, C. labiatum, Cylicocyclus nassatus, C. insigne, Cylicocercus catinatus, Cylicostephanus poculatus, C. hybridus, Cylicostephanus spp.(3), *Habronema muscae, Otobius megnini, Gastrophilus intestinalis, G. nasalis.*

105. *Parasitological Surveys in the Far East.* VI. *An Epidemiological Survey of Kyushu Island, Japan.* L. S. Ritchie, G. W. Hunter, III, C. Pan, M. Yokogawa and J. T. Szewczak. 406th Medical General Laboratory, Tokyo, Japan.

Kyushu, the southernmost island of Japan is comprised of 7 prefectures, 5 of which were surveyed during May and June 1948. The four areas investigated centered around the cities of Kurume, Kumamoto, Kagoshima and Beppu. Two to four population centers in and around these cities were examined. In addition to stool examinations, approximately 100 blood specimens for filaria were taken in each of two villages.

A total of 2073 individuals were examined; 92.6% of whom were found to harbor helminths and 33.2% protozoa. The helminths found and their incidences were: *Ascaris,* 74.6%; whipworm, 30.4%; hookworm, 50.2%; *Trichostrongylus* sp., 3.2%; *C. sinensis,* 3.3%; *M. yokogawai,* 4.9% and pinworm, 25.7%. The protozoa encountered and their incidences were: *E. histolytica,* 5.3%; *E. coli,* 19.9%; *E. nana,* 12.1%; *G. lamblia,* 6.0%. The incidence for *E. histolytica* in the Kurume area was only 1.8%.

S. japonicum which is endemic in the Kurume area was found in 72.9% and 60.2% of the population in the villages of Nagatoishi and Anrakuji respectively. These are the highest incidences yet encountered in Japan.

Of 103 blood specimens drawn at Yamanashi, a mountain village near Kumamoto where Japanese Health Officials had suspected filariasis, none were positive. At Kure village near Kagoshima, 21 of 108 individuals, 19.4% were positive for microfilariae of *W. bancrofti.*

106. *Parasitological Studies in the Far East.* VII. *An Epidemiological Survey in Southern Korea.* G. W. Hunter, III, L. S. Ritchie, I. C. Chang, W. D. Rolph, Jr., H. C. Mason and J. Szewczak. 406th Medical General Laboratory, Tokyo, Japan.

A survey for intestinal and blood parasites was undertaken in South Korea during July and August 1948. Specimens were collected, preserved and returned to the laboratory in Tokyo for examination. A total of 919 stool specimens were obtained from the following centers collectively: Seoul, Anyang, Ch'unch'on, Tae Jon, Kwangju, Mokp'o Taegu, Pusan, Cheju-Do. A rate of 94.2% was found for helminths and 33.4% for protozoa. Specific helminths and incidences were as follows: *Ascaris,* 82.4%; whipworm, 81.1%; (in 5 of the 9 areas the whipworm rate was higher than ascaris); hookworm, 46.5%; *Trichostrongylus* sp., 3.6%; *C. sinensis,* 7.1%, a rate of 41.1% occurred at Taegu; *M. yokogawai,* 1.6%; and pinworm by scotch tape swab was 20.2%. Protozoa and incidence were as follows: *E. histolytica,* 5%; *E. coli,* 27.1%; *E. nana,* 8.3% and *G. lamblia,* 3.5%.

A group of 334 American troops was also surveyed and the following parasitic rates were found: helminths collectively, 6.3%; protozoa collectively, 24%; *Ascaris,* 1.5%; whipworm, 0.9%; hookworm, 3.6%; *E. histolytica,* 4.5%; *E. coli,* 14.1%; *E. nana,* 9%; and *G. lamblia,* 3.3%. One or more parasites were found in 22.1% of the individuals who had been in Korea 3 months or less as compared to 30.3% of 248 who had been there over 3 months.

A series of 457 blood smears (thick and thin) were obtained from Koreans of which 3.3% were positive for malaria (*P. vivax*). Twenty three (23) examinations on the mainland of Korea for filariasis were negative, but two of 35 specimens from the island of Cheju-Do were positive for *Wuchereria malayi.*

107. *Gregarines Parasitic in Bermuda marine Crustaceans,* Gordon H. Ball, University of California, Los Angeles, California.

There is apparently no previous record of any examination of marine Crustaceans for gregarines in the Bermuda area. In the spring of 1949, the opportunity was presented of examining representative Crustaceans at The Bermuda Biological Station for Research. Gregarines were found in the digestive tracts of *Calappa flammea, Eupanopeus herbstii, Eupanopeus occidentalis, Mithrax forceps hirsutipes,* and *Pachygrapsus transversus.* On the basis of morphology, the parasites belong to previously undescribed species. Infection in some specimens was exceedingly heavy. Examination of 18 other species of Crustacea from the same area was negative for gregarines even though the hosts were found closely associated with the infected species.

It is of interest that an examination of 33 species of Hawaiian marine Crustaceans showed them all negative for gregarines except in the case of *Balanus eburneus,* which is very wide

in distribution. In some instances, the Hawaiian hosts belonged to the same genus as the infected species from Bermuda or from California.

108. *Species of Nematopsis in* Ostrea Virginica. Victor Sprague, Texas A and M Research Foundation and Young Harris College, Young Harris, Georgia.

Nematopsis ostrearum Prytherch, 1940, contains two species which commonly occur simultaneously as spore stages in American oysters but which differ significantly in size of resistant spore, distribution in the oyster, size of gymnospore, and crab host specificity. Small spores, formerly considered immature stages, are approximately 10 to 14 microns and are typically most concentrated in the mantle tissues. Their corresponding vegetative stages, as Prytherch demonstrated, occur in the mud crabs *Panopeus herbstii* and *Eurypanopeus depressus.* Present studies show that *Eurytium limosum,* under experimental conditions, harbors the same parasite. The diameter of the gymnospores, an important diagnostic feature, is approximately 3 or 4 microns. For this species, previously described in detail, the name *N. ostrearum* is reserved. Large spores, formerly considered the mature stages of *N. ostrearum* but now known to represent a distinct species, are approximately 10 by 19 microns and have special affinity for the oyster gills. Their corresponding vegetative stages occur in the stone crab *Menippe mercenaria.* The gymnospores are approximately 8 to 12 microns in diameter. Resistant spores and gymnospores, both being larger than those of any other known species, and the decapod host specificity distinguish this from any other *Nematopsis.* Since only the resistant spores were previously described, the species they represent is here removed from *N. ostrearum,* in which it was inadvertently included, and named *Nematopsis prytherchi* n. sp. A third type of *Nematopsis* spore, approximately 7 by 11 microns, in oyster gills may be a form of the latter species.

109. *The Life Cycle of* Diphyllobothrium latum (*Motion Picture*), M. S. Ferguson, Communicable Disease Center, Atlanta, Georgia.

This film depicts through cinematography and motion photomicrography of living material, and by animation, the life cycle of the broad tapeworm of man.

110. *Ultra-Rapid Demonstration of Blood Parasites by Thedane Blue Methods,* (*Motion Picture*). H. C. R. Simons, Chemical Biological Center, National Research Council, Washington, D. C.

111. *The Life of Dr. Howard Taylor Ricketts,* W. Malcolm Reid, Monmouth College, Monmouth, Illinois.

The family of Dr. Howard Taylor Ricketts has prepared a scrapbook giving an account of his life from early boyhood through his untimely death. Pages from his notebooks, manuscripts, correspondence with research workers throughout the world, and records of posthumous awards are included in facsimile together with all available photographs.

AMERICAN SOCIETY OF PARASITOLOGISTS

Thirty-Eighth Council Meeting, New Orleans, Louisiana
December 6, 1948

The meeting of the Council of the American Society of Parasitologists was called to order by President E. C. Faust at 7:30 PM, December 6, 1948, in the Roosevelt Hotel, New Orleans, Louisiana. Past-Presidents Harley J. Van Cleave, Asa Chandler, N. R. Stoll, H. E. Meleney, H. W. Stunkard, and the following members of the Council were present: E. C. Faust, C. B. Philip, H. W. Brown, R. M. Stabler, Paul D. Harwood, E. W. Price, Martin D. Young, T. W. M. Cameron, G. F. Otto and G. Robert Coatney.

The regular order of business was taken up.

I. Reports of Officers

1. *Secretary* (*H. W. Brown*): As of December 3, 1948, there were 658 members of the Society, of whom 586 lived within and 72 outside continental United States. Of these, 29 persons were delinquent for dues for 1948, leaving a net membership in good standing of 561 domestic and 68 foreign, or a total of 629 active members. This is the highest active membership which the Society has had. Forty-three persons were elected to membership during the calendar year 1948 to December 3, of whom 34 lived within and 9 outside continental United States. The names of 13 additional persons will be presented to the Council for election to the Society later in the meeting.

Since organization of the Society, 1465 persons have been elected to membership. The present active paid-up membership (629) represents 42.9% of the total number of electees during the 25 years of the Society's existence.

Richard P. Strong, President of the Society in 1927 and member of the Editorial Board from 1932–1939, passed away this year.

The report was accepted and placed on file.

2. *Treasurer* (*R. M. Stabler*): A summary of the Treasurer's report for the fiscal year 1948 (December 1, 1947, to December 1, 1948) follows:

a. The balance on hand as of Dec. 1, 1947 was $5,438.66.
b. The collections from all sources to Dec. 1, 1948 amounted to $8,844.12.
c. Total funds for the year, therefore, were $14,282.78.
d. Total expenditures were $9,884.12.
e. Total cash balance as of Dec. 1, 1948 is, therefore, $4,398.66.

It was pointed out that although the 1948 revenue exceeded that for 1947, and although expenses in the various offices were substantially the same, printing costs had risen sharply in the year 1948.

The report was accepted, subject to audit, and placed on file.

Audited and found correct by Auditing Committee:
(signed) Paul D. Harwood
(signed) Martin D. Young

II. Reports of Custodians and Committees

1. *Custodian of the Princeton Secretarial Fund* (*N. R. Stoll*): The report for the period December 1, 1947, to November 15, 1948, follows:

Savings bank balance, December 1, 1947	$1068.74
Interest credited	10.71
Savings bank balance Nov. 15, 1948	$1079.45

Balance on hand was confirmed by the First National Bank of Princeton, New Jersey, November 18, 1948.

The report was accepted and placed on file.

A motion to transfer this $1,079.45 to the Endowment Fund was carried.

2. *Chairman of the Editorial Committee* (*W. W. Cort, serving for H. W. Stunkard on leave*): It was reported that manuscripts were being received at a good rate, but increased printing costs have become a serious problem.

The report was accepted and placed on file.

3. *Custodian of Back Issues* (*G. F. Otto*): It was reported that during 1948 the Society sold nearly half as much stock of back issues as in all preceding eleven years. The $1500

made available from the general funds has been used to meet the costs of reproducing back issues, and proceeds from 1948 sales have been spent or are obligated. If sales continue as during 1948, it will be necessary to reproduce a few more issues to increase the number of complete sets.

4. *Auditing Committee (Paul D. Harwood and Martin D. Young)*: The reports of the Treasurer and of the Custodian of the Princeton Secretarial Fund were certified to be correct. The report of the Auditing Committee was accepted.

5. *Committee on Visual Instruction (M. S. Ferguson, Chairman, and W. Malcolm Reid)*: A preliminary report, outlining the program to be followed during 1949, was received and accepted.

III. Reports of Representatives

1. *To Council of A.A.A.S. (K. C. Kates and A. O. Foster)*: The report of the Society's representatives on the Council of the A.A.A.S. was received, and the Council voted to instruct them to vote yes on (1) the present distinction between members and fellows of the Association be abolished, (2) the present method of starring scientists in American Men of Science be abolished.

2. *To Governing Board of the American Institute of Biological Sciences (W. W. Cort)*: Dr. Cort's report on the American Institute of Biological Sciences was read and approved. Fourteen societies are now members of the A.I.B.S. Its governing board consists of one member from each society and four from the National Research Council. A plan for cooperative publishing of various society journals is under study and might result in savings to all societies. The A.I.B.S. will also serve as a clearing house for various information and meetings.

3. *To the Division of Biology and Agriculture of the National Research Council (E. W. Price)*: This report summarized the terms of the Fulbright Act (Public Law No. 584), which authorizes the Department of State to use a portion of the foreign currencies resulting from the sale of surplus property abroad for study, teaching, and research activities in foreign countries. The report was accepted and placed on file.

IV. New Business

1. *Election of New Members:* The following persons were elected by the Council to active membership in the Society: C. Julio Alvarez, Director of Diagnostic Laboratory, Vernaza Hospital, Guayquil, Equador; Sister Joseph Marie Armer, Professor of Biology, Incarnate Word College, San Antonio, Texas; Henry K. Beye, Assistant Professor and Field Director, Filariasis Project (Tahiti), University of Southern California Medical School; Laurent P. E. Choquette, Institute of Parasitology, MacDonald College, Quebec, Canada; Hernando Groot, Director of the Army Laboratories, Bogota, Colombia; Professor of Protozoology and Helminthology, Universidad Javeriana, Bogota; Eugene C. Haderlie, Teaching Assistant in Zoology, University of California; William G. Jahnes, Parasitologist and Malariologist, Army Medical School; Eugene N. Kozloff, Assistant Professor of Biology, Lewis and Clark College, Portland, Oregon; Max McCowen, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis 6, Indiana; Henry W. Robinson, graduate student at University of Southern California; Margaret A. Stirewalt, Parasitologist, Naval Medical Research Institute; Arthur H. Webb, Instructor in Bacteriology, Howard University Medical School, Washington 10, D. C.

2. *Place of Meeting in 1949:* It was voted that the Society meet in 1949 with the A.A.A.S. in New York City.

3. *Abstracts of the Annual Meeting:* The abstracts were discussed in the light of their cost (approximately $600 per year) and the greatly increasing cost of the Journal. It was brought out that for those who did not attend the meetings the Abstracts were an especially valuable source of information and that they are of great value in colleges with modest library facilities.

The motion "That the abstracts be eliminated and that the program be printed in the simplest form in the Journal with reprints for the meeting" was defeated. It was voted, in view of the importance of this matter to circularize the society by mail to ascertain the wishes of the membership in this matter.

(Note: 201 members have voted to continue publication of abstracts; 65 have voted to discontinue publication.)

4. *Amendments:*

a) *The Custodian of Back Issues* office, although approved by the Council in 1947, could not be created without Society action. The amendment creating this office was therefore to be presented to the membership at the 1948 general business meeting.

b) *An amendment to the constitution* "to raise the dues from $5.00 to $6.00 yearly,

which sum will include the A.I.B.S. dues, to take effect January, 1950" was recommended. This was to be announced at the 1948 business meeting of the Society and be voted on at the 1949 meeting.

c) *An amendment increasing the subscription rate* of the Journal to institutional subscribers and non-members from $6 to $7.50 annually beginning January 1, 1950, was recommended.

5. *Resolution:* It was voted to present to the Society N. R. Stoll's Resolution to the World Health Organization.

6. The petition by the Zoological Society of London for aid from the Society in the publication of the Zoological Record was tabled.

7. The request for permission by the Royal Society of Medicine to microfilm the Journal for distribution to war-stricken educational institutions was voted affirmatively.

8. Material sent to the Secretary by the Army outlining the advantages of the armed services for parasitologists was presented and discussed. The following motion was passed, to be sent to the A.I.B.S. for transmission to the Army, "the present policy of the army of recruiting professional biologists is referred to the American Institute of Biological Sciences by the American Society of Parasitologists with the comment that it cannot encourage parasitologists to select the Army as a career until scientists are assured a more equitable opportunity of achievement than is now apparent in the present organizational set-up."

9. *Nominations, Elections,* and *Appointments to Society Offices:*

a) *Nominations:* The following persons were nominated by the Council for the designated offices in the Society for 1949:

President, T. W. M. Cameron; Vice-president, W. H. Wright; Treasurer, R. M. Stabler (two years); Secretary (term runs through 1949); Custodian of Back Issues, G. F. Otto (three years); Councilors, J. C. Schwartzelder and J. T. Culbertson (1 year), G. L. Graham and L. A. Spindler (4 years).

b) *New Editorial Board* members elected were E. E. Byrd, W. L. Jellison, and G. R. Coatney.

c) *Endowment Fund:* It was voted to elect trustees of the endowment fund, this group to consist of N. R. Stoll and the President and Treasurer of the Society.

d) *Nominated for Honorary Foreign Member:* H. E. Shortt.

Respectfully submitted,
H. W. Brown, Secretary

TWENTY-THIRD ANNUAL GENERAL BUSINESS MEETING DECEMBER 8, 1948

The general business meeting of the Society was called to order by President E. C. Faust at 1:30 PM, following the annual luncheon at La Louisiane Restaurant, New Orleans, Louisiana.

1. A summary of the Secretary's report was given, calling attention to the new high in membership of 629, and the 43 new members for 1948.

2. The Treasurer's report was read for him by G. F. Otto, who called attention to the Society's cash balance of $4,398.66.

3. G. F. Otto reported on his year's activities as Custodian of Back Issues of the Journal of Parasitology. He has sold approximately $1,800 worth of back issues and plans to reproduce gradually all out-of-issue volumes.

4. H. W. Stunkard reported on the Journal of Parasitology and mentioned the difficulties in connection with increasing cost of publication.

5. G. F. Otto reported for W. W. Cort rather briefly on the American Institute of Biological Sciences, of which the American Society of Parasitologists is a member.

6. It was voted to establish officially the office of Custodian of Back Issues by an amendment to the constitution.

7. N. R. Stoll's resolution to the World Health Organization concerning the importance of parasitic infections in man was passed.

8. The members of the Society were informed that the Council had recommended an increase in the annual dues from $5 to $6 per year. This increase would include the A.I.B.S. dues of each member. The matter was called to the attention of the members, as the Society will vote on this issue at the 1949 business meeting. An amendment increasing the subscription rate of the Journal to institutional subscribers and non-members from $6 to $7.50 annually beginning January 1, 1950, was recommended.

9. The matter of the cost of publication of the annual abstracts of the meeting was mentioned and the members were informed that a mail vote would be taken to ascertain whether or not the membership wished to continue the publication of the abstracts. (Note: 201 members have voted to continue publication of abstracts; 65 have voted to discontinue publication.)

10. A motion was made and passed to have the Secretary write a letter of thanks to the local committee for the splendid arrangement of the program, and write letters to the other cooperating societies, thanking them for their splendid cooperation.

11. The officers and Council members of the American Society of Parasitologists nominated at the Thirty-Eighth Council meeting were eletced to their respective offices. The election of Dr. H. E. Shortt as Honorary Foreign Member was announced.

12. It was announced that the American Society of Parasitologists would meet with the A.A.A.S. in New York City in 1949.

The Society voted to adjourn at 2:45 PM.

Respectfully submitted,
H. W. Brown, Secretary

SOCIETY OFFICERS

AMERICAN SOCIETY OF PARASITOLOGISTS

Officers for 1949

THOMAS W. M. CAMERON, Macdonald College ..... *President*
WILLARD H. WRIGHT, National Institutes of Health ..... *Vice-President*
HAROLD W. BROWN, Columbia University ..... *Secretary*
ROBERT M. STABLER, Colorado College ..... *Treasurer*

Council Member Ex Officio[1]

HORACE W. STUNKARD, New York University ..... *Chairman, Editorial Committee*

Council Members at Large

(with date of expiration of term)

1952 G. L. GRAHAM, University of Pennsylvania
1952 L. A. SPINDLER, U. S. Bureau of Animal Industry
1951 PAUL D. HARWOOD, Dr. Hess & Clark, Inc.
1951 CLAY G. HUFF, Naval Medical Research Institute
1950 EMMETT W. PRICE, U. S. Bureau of Animal Industry
1950 MARTIN D. YOUNG, U. S. Public Health Service
1949 JOHN C. SWARTZWELDER, Louisiana State University
1949 JAMES T. CULBERTSON, National Institutes of Health

Representatives of the Society on the Council of the American Association for the Advancement of Science

(2-year terms expire 1949)

AUREL O. FOSTER KENNETH C. KATES

Representative of the Society on the Governing Board of the American Institute of Biological Sciences

W. W. CORT

Editorial Committee of the Journal of Parasitology

HORACE W. STUNKARD, *Chairman* ..... to serve until 1953
WILLIAM A. RILEY ..... to serve until 1953
JUSTIN M. ANDREWS ..... to serve until 1953

Editorial Board of the Journal of Parasitology

1952 E. E. BYRD, University of Georgia
1952 WILLIAM L. JELLISON, U. S. Public Health Service
1952 G. ROBERT COATNEY, National Institutes of Health
1951 ELERY R. BECKER, Iowa State College
1951 NORMAN R. STOLL, Rockefeller Institute for Medical Research
1951 GEORGE W. WHARTON, Duke University
1950 RICHARD J. PORTER, University of Michigan
1950 WILLIAM TRAGER, Rockefeller Institute for Medical Research
1950 ARTHUR C. WALTON, Knox College
1949 LLOYD E. ROZEBOOM, Johns Hopkins University
1949 WILLIAM W. CORT, Johns Hopkins University
1949 RAYMOND M. CABLE, Purdue University

Custodian of Back Issues

(3-year term expires 1951)

GILBERT F. OTTO

List of Former Officers

	President	*Vice-President*
1925	HENRY B. WARD*	SAMUEL T. DARLING*
1926	CHARLES W. STILES*	CHARLES A. KOFOID*
1927	RICHARD P. STRONG*	EDWIN LINTON*
1928	CHARLES A. KOFOID*	ROBERT HEGNER*

[1] Beginning in 1942, the Chairman, Editorial Committee, became ex officio member of Council.
* Deceased.

1929	Nathan A. Cobb*	George R. La Rue
1930	William W. Cort	Ernest Carroll Faust
1931	William A. Riley	Asa C. Chandler
1932	Maurice C. Hall*	William H. Taliaferro
1933	William H. Taliaferro	Fred C. Bishopp
1934	Ernest E. Tyzzer	James C. Ackert
1935	Charles F. Craig	Harley J. Van Cleave
1936	Robert Hegner*	William B. Herms*
1937	George R. LaRue	David H. Wenrich
1938	Fred C. Bishopp	Elery R. Becker
1939	Horace W. Stunkard	Henry E. Meleney
1940	David H. Wenrich	Gotthold Steiner
1941	James E. Ackert	Justin Andrews
1942	Henry E. Meleney	Rudolf W. Glaser*
1943	Henry E. Meleney	Rudolf W. Glaser*
1944	Henry E. Ewing	Benjamin Schwartz
1945	Asa C. Chandler	Donald L. Augustine
1946	Norman R. Stoll	Harold Kirby, Jr.
1947	Harley J. Van Cleave	Clay G. Huff
1948	Ernest Carrol Faust	Cornelius B. Philip

Secretary-Treasurer

William W. Cort	1925; 1926; 1927; 1928; 1929
Norman R. Stoll	1930; 1931; 1932

Secretary

Horace W. Stunkard	1933–34; 1935–36; 1937
Oliver R. McCoy	1938–39; 1940–41; 1942
James T. Culbertson	1942–43; 1944–45; 1946–47
Harold W. Brown	1948–

Treasurer

Austin Andrews	1933–34; 1935–36
Gilbert F. Otto	1937–38; 1939–40; 1944
L. E. Rozeboom	1941–42; 1943–44
Robert M. Stabler	1945–46; 1947–48; 1949–

Council Members at Large

Paul Bartsch	1925–28	L. R. Cleveland	1931
Fred C. Bishopp	1925–28; 1929–30	W. W. Cort	1931–34; 1935–38
Robert Hegner*	1925–27	H. E. Ewing	1931–32
Charles A. Kofoid*	1925	Ernest C. Faust	1931–34; 1938–41
B. H. Ransom*	1925	John F. Kessel	1932–35
William A. Riley	1925–26; 1928–30	D. H. Wenrich	1932–35; 1936
Charles W. Stiles*	1925; 1929–32	H. E. Meleney	1933–36
Ernest E. Tyzzer	1925–26	Norman R. Stoll	1933–36; 1937–47; 48
Maurice C. Hall*	1926–29	Eloise B. Cram	1934–37
Wilson G. Smillie	1926–27	Wilbur A. Sawyer	1934–37
Henry B. Ward*	1926–29	James E. Ackert	1935–38
Franklin D. Barker*	1927–30	Earl C. O'Roke	1936–39
J. H. St. John*	1927–28	Justin Andrews	1937–40
W. H. Taliaferro	1928–31	Willard H. Wright	1942–45; 1946–48
Asa C. Chandler	1929–30; 1936–39	Harold W. Brown	1944–47
Harley J. Van Cleave	1938–41	Harold W. Manter	1944–46
Elery R. Becker	1939–43	G. Robert Coatney	1945–48
Emmett W. Price	1939–43; 1944–46; 1947	T. W. M. Cameron	1946–48
Clay G. Huff	1940–43; 1944–46; 1948	Martin D. Young	1947
Horace W. Stunkard	1940–43	Cornelius B. Philip	1947
Donald L. Augustine	1941–44	Paul D. Harwood	1948
Raymond M. Cable	1942–45	John C. Swartzwelder	1949
Gilbert F. Otto	1942–44; 1945–48	James T. Culbertson	1949
W. B. Herms*	1930–33	G. L. Graham	1949
Benjamin Schwartz	1930–33	L. A. Spindler	1949

Editorial Committee of the Journal of Parasitology

William W. Cort, *Chairman*	1932–37; 1948
Robert Hegner*	1932–34
Francis M. Root*	1932–34
Norman R. Stoll, *Chairman*	1938–42; 1943
Horace W. Stunkard, *Chairman*	1944–47; 1949–
William A. Riley	1934–37; 1938–42; 1943; 1944–48; 1949–
William H. Taliaferro	1934–37; 1938–42; 1943–
David H. Wenrich	1944–48

Editorial Board of the Journal of Parasitology

Charles F. Craig	1932–33; 1934–37
Maurice C. Hall*	1932–33
Henry B. Ward*	1932–33
Asa C. Chandler	1932–34; 1935–38; 1939–42
Charles A. Kofoid*	1932–34; 1935–38
William A. Riley	1932–34
W. H. Taliaferro	1932–34
James E. Ackert	1932–35
Richard P. Strong*	1932–35; 1936–39
Fred C. Bishopp	1932–36
George P. LaRue	1932–36
David H. Wenrich	1932–36; 1938–41
Ernest C. Faust	1933–37
Benjamin Schwartz	1933–37; 1938–41; 1942–45
Elery R. Becker	1934–35; 1936–39; 1948
Robert Matheson	1935–38
Oliver R. McCoy	1936–39
Henry E. Ewing	1937–40
John F. Kessel	1937–40
Harley J. Van Cleave	1937–40
William W. Cort	1938–41; 1942–45; 1946–49
Cornelius B. Philip	1939–42
Ernest E. Tyzzer	1939–42
Harold W. Brown	1940–43
Harold W. Manter	1940–43
Reginald D. Manwell	1940–43
Richard P. Hall	1941–44
E. Harold Hinman	1941–44
Justus F. Mueller	1941–44
Harold Kirby	1942–45
Benjamin G. Chitwood	1943–46
Pincus P. Levine	1943–46
Rudolf Glaser*	1943–46
Lowell T. Coggeshall	1944–47
John T. Lucker	1944–47
Norman R. Stoll	1944–47; 1948
William L. Jellison	1945–48; 1949
Charles W. Rees	1945–48
Lloyd A. Spindler	1945–48
Raymond M. Cable	1946–49
Lloyd E. Rozeboom	1946–49
Richard J. Porter	1947
William Trager	1947
Arthur C. Walton	1947
George W. Wharton	1948
E. E. Byrd	1949
G. Robert Coatney	1949

List of Meeting Places

1925	Kansas City	1933	Boston	1941	Dallas
1926	Philadelphia	1934	Pittsburgh	1942	(New York, cancelled)
1927	Nashville	1935	St. Louis	1943	(No meeting)
1928	New York	1936	Atlantic City	1944	Cleveland
1929	Des Moines	1937	Indianapolis	1945	St. Louis
1930	Cleveland	1938	Richmond	1946	Boston
1931	New Orleans	1939	Columbus	1947	Chicago
1932	Atlantic City	1940	Philadelphia	1948	New Orleans

* Deceased.

IN MEMORIAM

MITCHEL CARROLL

ROBERT C. RHODES

WILLIAM B. HERMS

AMERICAN SOCIETY OF PARASITOLOGISTS

LIST OF MEMBERS ELECTED

Since October 1, 1948[1]

[1] To November 1, 1949. Members elected between November 1, 1947 and October 1, 1948 are listed in vol. 34 (supplement) : 43; the last preceding list of members was published in the Journal (1947) vol. 33 (supplement) : 35.

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WOLFTHAL, ABRAHAM, B.S., B.A. 63 Barrows Avenue, Willimantic, Connecticut.

WONG, CHESTER, B.A. 312 North Boyle Avenue, Los Angles 33, California.